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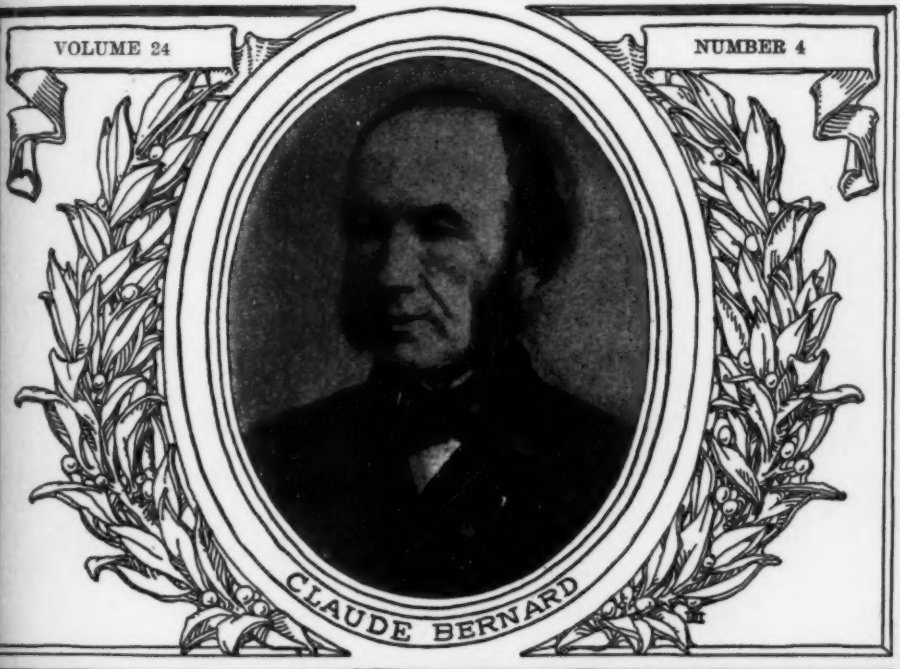

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WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA, PA.

THE EFFECT OF GLUCOSE AND SUCROSE ON THE RESPIRATORY QUOTIENT AND MUSCULAR EFFICIENCY OF EXERCISE

MARGARET WRIGHTINGTON

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TWO FIGURES

(Received for publication April 27, 1942)

The controversial question of just how fats and carbohydrates are utilized for muscular energy has been studied from many angles. One of the most important of these is the interpretation of the respiratory quotients obtained during exercise. The specific effects on the respiratory quotient following the ingestion of various sugars were first studied by Carpenter and his associates (Carpenter and Fox, '31 a, b; Carpenter and Lee, '38). The resting R.Q. was increased by fructose to a much greater extent than by glucose, even after a correction was made for the greater lactic acid accumulation caused by fructose. It was suggested that the higher R.Q. was due to the conversion of fructose to fat. The total work R.Q. was also increased more by fructose than by glucose. However, more glucose than fructose was used in the excess metabolism occasioned by work. The apparent increment in the work R.Q. caused by fructose was probably due only to this sugar's conversion to fat. Carpenter and his associates were able to find no significant difference in the efficiency of the work after the ingestion of sugars.

Haggard and Greenberg ('35) obtained R.Q. values after the ingestion of sucrose and glucose which were very similar

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to those of Carpenter; the greater increase found for sucrose was probably due to its fructose component. In contrast to Carpenter's findings, however, they reported that the mechanical efficiency of exercise paralleled very closely the changes in the R.Q. after each sugar.

Bachmann, Haldi and assistants ('37; Haldi and Bachmann, '37; Haldi, Bachmann and Ensor, '38) confirmed the work of Carpenter and his associates in every respect. They were unable to find any correlation between efficiency and R.Q.

In the work reported herewith, the effects of glucose and sucrose on the resting and work R.Q.'s and on the muscular efficiencies are found to confirm the conclusions of Carpenter. In addition, an interesting relationship between resting and work R.Q.'s is described.

METHODS

The present work was done on a Krogh bicycle ergometer. The subject pedalled at a uniform rate for 10 minutes, accomplishing 15 kilo-calories of work. The respiratory exchange was obtained with a Benedict Universal machine which was carefully tested for leaks before every run and checked for accuracy at intervals with an alcohol flame. The alcohol R.Q.'s ranged from 0.67 to 0.68 (theoretical — 0.667). Most of the experiments were done on one young male student about 7 hours after breakfast.[1] Data for an initial rest period of 7 minutes were obtained while he sat on the bicycle. After the subsequent 10-minute work period, he stopped pedalling but continued to breathe into the system for 15 to 20 minutes until recovery was complete, as indicated by the return of the alveolar CO_2 concentration, pulse and respiration rates to rest levels. Fifty grams (unless otherwise indicated) of

[1] No attempt was made to control the diet, since one of the points of interest in this study was the relationship of the muscular efficiency to different R.Q.'s. The erratic eating habits of this subject (his breakfasts, which he occasionally missed completely, varied from sugar cookies to the conventional large breakfast) were reflected in the great variability of the baseline R.Q.'s. Notes made at the time showed that the R.Q.'s varied in the directions expected from the quality and quantity of his breakfasts.

sucrose or an equicaloric amount of glucose in 400 cc. of water containing the juice of one lemon were then ingested. After 30 minutes he returned to the bicycle, sat through another rest period, and then was observed through a period of work and recovery.

Nitrogen determinations in the urine were omitted as they were shown in preliminary experiments to alter the R.Q.'s only in the third decimal place.

The glucose was given in the form of a spray-dried corn syrup [2] and a more hydrolyzed form of corn syrup.[3]

RESULTS

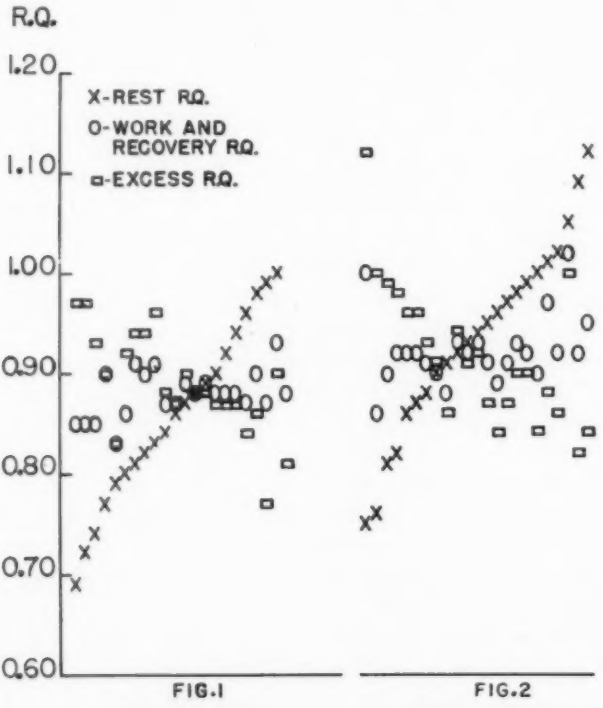
The respiratory quotient

The variation in the baseline R.Q.'s (6–8 hours after breakfast) from 0.69 to 1.00 shows that the subjects were not in a uniform state; however, there is presented the opportunity to study the metabolism of work over a wide range of rest R.Q. values. The average R.Q. for 43 baseline rest periods is 0.87; for the total work and recovery, 0.88; and for the excess metabolism due to the work alone, 0.89. In figure 1, the resting R.Q.'s are arranged in numerical order, in this way falling along a regularly rising curve from the left to the right of the figure. The work and recovery R.Q.'s lie in a narrow horizontal band around the average value of 0.88. It will be seen that the work and recovery R.Q.'s are higher than their corresponding rest R.Q.'s when the latter are less than 0.88. Work and recovery R.Q.'s corresponding to rest R.Q.'s of 0.88 and 0.89 are numerically identical, and above this they are lower than their rest R.Q.'s. The excess R.Q.'s vary from their corresponding rest R.Q.'s in a similar manner, but to a greater extent, thus falling along a descending curve as the rest R.Q.'s increase. Again, in the center of the figure where the rest R.Q.'s are 0.88 and 0.89, the excess values are very close to these numbers. The

[2] This is not all preformed glucose, but quickly yields only glucose in digestion.
[3] "Sweetose" supplied by the Staley Manufacturing Company.

greatest differences occur at the two extremes of the rest R.Q. scale.

The results obtained 30 minutes after the ingestion of the sugars are similarly arranged in figure 2. The average value for the rest R.Q. is 0.93; for the work and recovery R.Q., 0.92; and for the excess R.Q., 0.91. As in figure 1, the R.Q. values fall into a similar pattern, in which the same relations exist between rest, work and recovery, and excess



R.Q.'s before sugar. R.Q.'s after sugar.

The averages of the work and recovery and excess R.Q.'s lie in a vertical line with their corresponding rest R.Q.'s. R.Q.'s corresponding to rest values from 0.80 to 0.96 in figure 1 and from 0.86 to 0.99 in figure 2 are averages of from 2 to 4 runs. Some values obtained in other experiments not included in the tables have been added to this chart.

R.Q.'s as were found in the baseline experiments. As expected, the pattern is placed at a higher level in the R.Q. scale. In this case, the three R.Q.'s coincide at rest levels of 0.92–0.93.

The effect of different sugars on the R.Q.

Sucrose (table 1) increased the rest R.Q. from an average of 0.84 to 0.92 — a difference of 0.08; whereas, glucose (table 2) elicited a rise of only 0.01. To determine the amounts of

TABLE 1

The R.Q. and per cent net efficiency before and after sucrose.

SUBJECT	DATE	BEFORE SUCROSE				AFTER 50 GM. SUCROSE			
		R.Q.			Per cent net efficiency	R.Q.			Per cent net efficiency
		Rest	Work and recovery	Excess		Rest	Work and recovery	Excess	
S.W.	12/13	.90	.82	.76	19.77	.91	.86	.82	24.47
S.W.	12/17	.94	.81	.79	19.13	.99	.93	.88	20.34
G.D.	2/14	.94	.91	.88	19.87	.98	.93	.89	19.17
G.D.	2/19	.72	.85	.95	20.08	.76	.88	1.00	21.17
G.D.	2/29					.81	.90	.99	21.32
G.D.	2/21	.74	.86	.93	19.31	.93	.90	.87	19.40
G.D.	2/22	.72	.87	1.01	22.87	.93	.94	.94	20.90
G.D.	2/26	.81	.96	1.04	20.88	.87	.95	1.03	21.17
R.K.	2/27	.94	.91	.89	20.61	.92	.93	.93	21.81
R.K.	2/28	.84	.87	.88	19.47	.93	.91	.91	20.45
R.K.	2/29	.88	.84	.81	19.73	1.04	1.02	1.00	21.59
Average		.84	.87	.89	20.18	.92	.92	.93	21.07
		Net increase ("after" minus "before sugar")				.08	.05	.04	.89
		Minimum difference required for statistical significance							1.04

these sugars catabolized during work, the excess R.Q. is corrected for the increase due to the sugar alone, that is, by subtracting from the excess R.Q., the difference between the pre- and post-sugar resting R.Q.'s. When the excess R.Q. after sucrose is corrected in this way, the result, 0.85, is even lower than the pre-sugar R.Q. With glucose, however, this calculation gives a corrected excess R.Q. of 0.92, which is clearly higher than the control pre-sugar value. This indicates

TABLE 2

The R.Q. and per cent net efficiency before and after glucose.

SUBJECT R.K. THROUGHOUT		BEFORE GLUCOSE				AFTER GLUCOSE			
Date	Syrup	Rest	R.Q. Work and recovery	Excess	Per cent net efficiency	Rest	R.Q. Work and recovery	Excess	Per cent net efficiency
2/28	Sweetose	.83	.97	1.06	20.28				
3/ 1	50 gm.	.90	.87	.86	20.63	.88	.95	1.00	21.75
3/ 4	50 gm.	.97	.90	.86	21.33	.96	.92	.90	21.27
3/ 5	50 gm.	.86	.83	.82	20.91	.86	.92	.97	21.47
3/ 6	50 gm.	.79	.83	.85	20.35	.82	.92	.98	21.23
4/26	50 gm.	.83	.84	.88	20.62	.88	.90	.91	20.15
4/29	50 gm.	.98	.88	.77	21.11	1.01	.97	.88	20.36
5/ 1	102 gm.	.92	.88	.87	20.47	.94	.93	.92	21.14
5/ 2	102 gm.	.89	.88	.88	20.40	.88	.89	.90	21.01
5/ 3	102 gm.	.96	.87	.85	21.43	.87	.91	.93	20.54
5/ 8	Corn syrup	.86	.87	.86	21.29	.91	.90	.89	22.56
5/ 9	50 gm.	.94	.88	.84	21.50	.86	.91	.94	20.86
5/12	50 gm.	.86	.88	.90	21.93	.93	.92	.91	21.69
5/15	50 gm.	.87	.86	.86	21.00	.92	.92	.92	21.58
Average		.89	.87	.87	21.09	.90	.92	.93	21.20
Net increase ("after" minus "before sugar")						.01	.05	.06	.11
Difference required for significance									.73

that more glucose than sucrose is metabolized during work, a fact which is not apparent from a comparison of the uncorrected excess R.Q.'s, in which sucrose is higher.

Muscular efficiency

The percentage net efficiencies, obtained by dividing the heat of the mechanical work done by the excess energy caused by this work, are included in tables 1 and 2. Although there is an increase after both sugars, the increase after sucrose narrowly misses significance, whereas the increase after glucose is clearly not significant.

DISCUSSION

Krogh and Lindhard ('20) have suggested that an interconversion of fats and carbohydrates occurs, depending on

their relatively available quantities in the body. When the supply of carbohydrate is in excess of that of fat, fat is formed from carbohydrates. This occurs at R.Q.'s above 0.90. The reverse occurs when the quotient is below 0.80. During work, this anabolic process continues unaltered or may possibly be reduced; at least, it is not increased in proportion to the increased catabolism. Its relative effect on the total R.Q. of work is diminished, causing the R.Q. to rise when it is low during the pre-work rest period, and fall when it is high at rest. The R.Q. arrangement shown in figures 1 and 2 would seem to be a good illustration of this hypothesis. As most of the baseline R.Q.'s cluster around 0.88 (fig. 1), it might be said that the normal equilibrium in this particular nutritive state occurs at this value, when, according to the Zuntz-Schumburg tables, the proportion of carbohydrate to fat burned would be 59 to 41. When either fat or carbohydrate is in excess, the equilibrium is destroyed and in an attempt to restore the balance, the rest R.Q. will be raised or lowered according to the direction of the fat $\leftrightarrows$ carbohydrate conversion. During work, when the catabolic phase is stressed, the work R.Q. value should approach the equilibrium value of 0.88. Such changes were observed. The work and recovery values as shown here all lie very close to 0.88, seldom going over 0.91 or below 0.85. The excess R.Q.'s, however, should vary in exactly the reverse direction from the rest R.Q.'s. This was also observed and can be seen from the descending trend from left to right in figure 1. The excess R.Q.'s corresponding to rest R.Q.'s of 0.87–0.89 are numerically identical. But from the indirect method of calculating this value $\frac{\text{Work } CO_2 - \text{Rest } CO_2}{\text{Work } O_2 - \text{Rest } O_2}$ in cases where there is a large change, either increase or decrease, from the rest R.Q. to a value of 0.88 in the work and recovery period, there will be, of necessity, an even larger change in the same direction in the excess R.Q.

Thirty minutes after the ingestion of sugar (fig. 2) a nutritional state exists in which all of the relations between the various R.Q.'s found in the baseline experiments are essentially repeated. However, the equilibrium is now established

at an R.Q. of about 0.92, at which 73% carbohydrate to 26% fat is burned.

The results obtained on the effect of various sugars on the R.Q. are in agreement with those of the workers cited above. There is a greater rise in R.Q. after sucrose, due to its fructose component, which according to their interpretation indicates conversion of this sugar to fat. It is also shown that more glucose than sucrose is metabolized during work.

Carpenter and Fox and others, and Bachmann, Haldi and associates, were unable to find any change in muscular efficiency after ingestion of various sugars, regardless of the quantities taken or the time intervals after ingestion. The latter workers repeated Haggard and Greenberg's experiments under nearly identical circumstances, but were unable to confirm the striking and apparently consistent increases reported in that investigation. The data in this paper add confirmation to the reports of Carpenter, and Bachmann and Haldi, since no statistically significant increases in the mechanical efficiency were observed following the ingestion of sugars.

SUMMARY

1. The rest R.Q.'s 6–8 hours after the last meal with no control of the diet vary over a wide range, from 0.69 to 1.00. When the rest R.Q.'s are arranged numerically, the work and recovery, and excess R.Q. values increase if their corresponding rest R.Q. is below 0.88 for pre-sugar experiments or below 0.92 for post-sugar experiments, and decrease if their rest R.Q. is above these figures. These data are interpreted by the hypothesis that when fat is in excess it is converted to carbohydrate and stored, and when carbohydrate is preponderant, the reverse occurs.

2. Sucrose, probably because of the conversion of its fructose component to fat, raises the rest R.Q. higher than do the glucose corn syrups.

3. Following the ingestion of glucose and of sucrose, the excess R.Q.'s, when corrected for the increase due to sugar

alone, show that more glucose than sucrose is metabolized during work.

4. No significant changes in muscular efficiency occur after the ingestion of either of the sugars.

ACKNOWLEDGMENT

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THE THIAMINE REQUIREMENT OF THE ALBINO RAT AS INFLUENCED BY THE SUBSTITUTION OF PROTEIN FOR CARBOHYDRATE IN THE DIET[1]

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(Received for publication January 22, 1942)

The first indication that the thiamine requirement might depend on the nature of the diet came in 1914 when Funk, and Braddon and Cooper discovered that diets high in carbohydrate hastened the outbreak of beriberi. More recently the problem has received the attention of Roche ('31), Peters ('36), Banerji and Harris ('39), and Arnold and Elvehjem ('39). Numerous workers, beginning with Platt and Lu ('35), have used the increases in both blood and urinary pyruvate and bisulphite-binding substances as indices of deranged carbohydrate metabolism in thiamine deficiency. The most recent studies have been those of Shils, Day and McCollum ('41), Harper and Deuel ('41), and Harper ('42) on various factors influencing the urinary excretion of these substances.

The relationship between thiamine and the protein of the diet has received considerable attention, and yet no one, except Funk and his colleagues (Funk, Collazo and Kaczmarek, '25), has suggested a role for protein analogous to the "sparing" role described by Evans and Lepkovsky ('28) for fat. Although Sherman and Gloy ('27), Hogan and Pilcher ('33) and Guerrant and Dutcher ('34) all reported that variations in the protein content of the diet had no effect on the vitamin B_1

[1] Authorized for publication on January 9, 1942, as paper no. 1078 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

requirement, Evans, Lepkovsky and Murphy ('34) found that for fat to exert its optimum sparing action, the protein content of the diet had to be high. More recently it has been demonstrated that the addition of vitamin B_1 to the vitamin B-complex-free diet of rats favors the free-selection of carbohydrate, and to a lesser but almost equally significant degree, the free-selection of protein (Richter and Hawkes, '41). It is probable, therefore, that thiamine participates in the metabolism of the proteins to the extent of the glycogenic amino acids which they contain.

The present investigation is concerned with the role of carbohydrate and protein in relation to the thiamine requirement of the adult, male albino rat. It is intended that the feeding of a high protein diet (64% casein) to rats which had previously received a high carbohydrate diet (64% sucrose) should reveal a decrease in the thiamine requirement. The change in the requirement should parallel the decrease in the glycogenic constituents of the diet.

EXPERIMENTAL

The adult, male albino rats used in this experiment (Wistar Institute strain) were taken at random from among a large number of stock colony weanlings and maintained on commercial dog food [2] until approximately 9 months of age. They were then separated and placed in cylindrical wire-mesh metabolism cages in preparation for the experiment. The final distribution was not wholly a random one in that the six smallest animals were selected to make up the deficient and normal control groups.

The 24-hour urine collections were started at exactly 9 A.M., at which time the metabolism cages were placed on an 8-inch funnel into which a 7-inch circular screen of a mesh sufficiently small to catch the fecal pellets had been placed. The receiving vessel consisted of a 250 cc. Erlenmeyer flask. The trichloracetic acid (15 cc. of a 10% solution) used as a preservative was poured down the sides of the funnel so as to

[2] Purina dog chow.

coat the inner wall with acid. The small amount of urine that was caught in the meshes of the fecal-retaining screen dried there without the benefit of a preservative. The funnel and the screen were washed with approximately 100 cc. of distilled water at the end of the collection period and the combined urine, preservative, and washings were filtered superficially through cloth or coarse filter paper to remove the hair and spilled feed and then made up to a volume of 250 cc.

TABLE 1

Composition of diets used.

INGREDIENTS	HIGH CARBOHYDRATE [1]	HIGH PROTEIN
Basal mixture, in %:		
Sucrose [2]	64.0	19.6
Casein [3]	20.0	64.0
Hydrogenated cottonseed oil [4]	9.89	9.88
Cellulose flour	2.85	3.04
Salts [5]	3.00	3.20
Supplements (per 10 kg. of basal mixture):		
Viosterol in oil (10,000 I.U. per gm. oil) (gm.)	3.333	3.589
Carotene in oil (3.69 mg. per gm. oil) (gm.)	8.000	8.615
Riboflavin (mg.)	50.0	53.9
Pyridoxine (mg.)	50.0	53.9
Ca pantothenate (mg.)	24.0	25.9
Lecithin (gm.)	15.00	16.14

[1] Gross energy value 4.7 calories per gram.
[2] Moisture content of: sucrose, 0.02%; casein, 4.09%.
[3] Labco, vitamin-free, from Borden Company.
[4] Crisco (10% diminished by oil in supplements).
[5] Hubbell, Mendel and Wakeman ('38).

The urinary pyruvate was determined by the colorimetric method of Lu ('39) with the slight modification that a compound Corning filter (nos. 502, 3.95 mm.; 430, 4.00 mm.; 315, 3.00 mm.) was used instead of the Wratten no. 62. One cubic centimeter of the collected urine that had been made up to a volume of 250 cc. was used for each duplicate determination. The composition of the diets is given in table 1.

Preliminary studies made with the high carbohydrate diet (table 1) revealed that:

(1) A 10% solution of trichloracetic acid, when used as a preservative, prevented the loss of a small amount of pyruvate during the 24-hour collection period. The freshly excreted urines of two rats were so divided that duplicate determinations for pyruvic acid could be made after the urines had been exposed to the atmosphere for 24 hours with and without trichloracetic acid. In both instances the untreated samples averaged 3% less pyruvic acid than did the treated samples.

(2) The normal excretion of two rats receiving 16 gm. of the diet daily was unchanged at the end of 4 weeks. The rats were apparently able to utilize the synthetic diet in a normal manner and the diet contained no unknown substances which might have surreptitiously influenced the pyruvate excretion.

(3) The thiamine requirement of adult, male rats (347 to 374 gm.) receiving 16 gm. of the diet was somewhat above 20 μg. of thiamine per day. Nine rats were divided into three groups of three rats each and given 10, 15 and 20 μg. of thiamine daily. All animals showed increased pyruvate excretions.

Eighteen mature, male rats (maintained at 26–30°C.) were used for the main experiment, of which three served as deficient controls (nos. 1–3, weighing 321 to 325 gm.), and three as normal controls (nos. 4–6; initial weight 308 to 339 gm.; final weight 375 to 396 gm.). Normal urinary pyruvate values were first determined for all rats following a week's feeding of the high carbohydrate, thiamine-supplemented diet (14 gm. containing 5 μg. of thiamine per gram of diet). The deficient controls were then transferred to a high carbohydrate diet wholly deficient in thiamine, and rats 7 to 18 (initial weight 332 to 414 gm.; final weight 384–437 gm.) were divided into four groups of three rats each. Following the 2-day depletion period during which they were given the deficient diet, the four groups were then supplemented with 18, 20, 22 and 24 μg. of thiamine daily. It soon became apparent that 24 μg. was not sufficient to meet the needs of the three animals on the highest

plane of intake and the intake of all groups was increased 8 μg. per day to 26, 28, 30 and 32 μg., respectively. In approximately 2 weeks it was clear that individual differences were greater than was first supposed, and the group plan was abandoned. From that time on each animal was treated individually.

The results obtained with the high carbohydrate diet are presented in table 2. The individual thiamine requirements in the last column are all estimated to be within at least 2 μg. of the true value. In only two cases (rats 10 and 13) did the final pyruvate values decrease to what the initial values had been. The final requirement for each of the remaining animals was estimated on the basis of former responses to the increased administration of thiamine, as well as on the basis of the performance of the group as a whole. The factors of time and the amount of available diet prevented the extension of the experiment for another 2 or 4 weeks.

The daily thiamine requirement values for rats 12 and 18 were not recorded (rat 18 being destroyed) because they refused food during the last week and during the final collection period. Not only has it been shown that the quantity of food metabolized influences the excretion of pyruvic acid (Shils, Day and McCollum, '41), but since the vitamin supplement was weighed separately and added to the diet each day, for which purpose casein served as a vehicle (1 mg. of casein contained 1 μg. of thiamine), there was no way of determining whether the supplement had been consumed.

The high protein diet on analysis was found to contain 5.485 calories per gram. Since the plan of the experiments called for isocaloric or near-isocaloric feeding, the high protein diet was first administered at the 12-gm. level of intake and not at the 13-gm. level as had been planned before the energy analysis was made. The immediate and numerous food refusals, which prompted a lowering of the food intake in order to stimulate the appetite, as well as the small but significant gains made when the animals were receiving the high carbohydrate diet in an isocaloric amount, suggested one conclusion:

TABLE 2

High carbohydrate diet:

The relation of pyruvate excretion to the intake of thiamine.

PERIOD	1		2			3		4		
Rat No.	B_1/day	P.A.[1] in 24 hrs.	B_1/day		P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day		P.A. in 24 hrs.
	7 days		2 days	5 days		7 days		4 days	3 days	
	μg.	mg.	μg.	μg.	mg.	μg.	mg.	μg.	μg.	mg.
1	70	1.8	0	0	2.7 [2]	0 [3]	6.4 [2]	0 [3]	0 [3]	7.9 [2]
2	70	1.8	0	0	2.1	0	6.7 [2]	0 [3]	0 [3]	4.9 [2]
3	70	2.0	0	0	2.9	0	5.5	0 [3]	0 [3]	7.6 [2]
4	70	1.7	70	70	1.9	70	1.9	70	70	2.0
5	70	2.3	70	70	2.4	70	2.6	70	70	2.7
6	70	2.4	70	70	2.6	70	3.1	70	70	2.9
7	70	2.5	0	18	3.1	18	3.7	18	26	3.3
8	70	2.7	0	18	3.2	18	5.0	18	26	4.5
9	70	2.6	0	18	3.3	18	4.4	18	26	4.4
10	70	1.8	0	20	2.8	20	3.2	20	28	3.2
11	70	2.1	0	20	2.8	20	3.8	20	28	3.7
12	70	2.0	0	20	2.4	20	3.2	20	28	2.9
13	70	2.5	0	22	3.5	22	3.4	22	30	3.8
14	70	3.4	0	22	3.9	22	4.6	22	30	5.2
15	70	1.6 [2]	0	22	3.5	22	3.8	22	30	3.9
16	70	2.1	0	24	2.8	24	3.2	24	32	3.1
17	70	2.7	0	24	2.8	24	4.0	24	32	4.3
18	70	3.4	0	24	4.1	24	5.3	24	32	6.6

PERIOD	5		6		7		8		DAILY B_1 REQUIREMENT
Rat No.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	
	7 days		7 days		7 days		7 days		
	μg.	mg.	μg.	mg.	μg.	mg.	μg.	mg.	μg.
4	70	1.9	70	2.1	70 [2]	1.8	70 [3]	2.1	
5	70	2.3	70	2.5	70	2.2	70	2.4	
6	70	2.9	70	2.6	70	2.4	70	2.8	
7	26	3.0	26	3.7	30	3.4	30	2.8	30
8	26	4.6	26	4.9	32	4.2	32	3.9	34
9	26	4.4	26	4.8	32	3.6	32	4.3	36
10	28	3.2	28	3.7	32	3.2	32	2.5	32
11	28	4.7	28	3.4	30	3.4	30	3.1	32
12	28	2.9	28	3.1	32	2.7	32 [3]	(2.1) [2]	..
13	30	2.8	30	2.8	28	2.6	28	2.7	28
14	30	5.0	30	4.8	32	4.3	32	4.1	34
15	30	3.8	30	3.6	30	3.5	30	3.0	32
16	32	3.2	32	3.6	34	2.7	34	2.8	36
17	32	4.3	32	3.8	32	3.2	32	3.5	34
18	32	5.6	32 [3]	4.6 [2]	30 [3]	3.8 [2]	30 [3]	(4.7) [2]	..

Average = 33

(Coefficient of variation = 8%)

[1] Pyruvic acid (P.A.) determined on urine of last 24 hours in each period.

[2] Food refused during urine collection.

[3] Food refused at least once during period indicated; but not during urine collection, unless indicated.

that the 12-gm. level of intake was too high. Subsequently, the diet was fed at the 11-gm. level until the experiment ended. Thus, the animals received 60.3 calories per day when on the high protein diet as compared to 65.8 calories per day when on the high carbohydrate diet.

The daily thiamine supplements that were initially administered to the assay animals on the high protein diet were estimated with the aid of both the previous requirements on the high carbohydrate diet and the ratio of glycogenic substances contained in the two diets. The casein was held to be approximately 50% glycogenic on the basis of Schmidt's ('38) list of glycogenic amino acids, and Hawk and Bergeim's ('37) analysis of casein as adapted from Mitchell and Hamilton. The hydrogenated cottonseed oil [3] was considered to be at least 10% glycogenic by virtue of the glycerol it contained. It was estimated that the 12 gm. of the high protein diet initially fed would yield 6.17 gm. of glycogenic substances as compared to 10.45 gm. contained in the 14 gm. of the high carbohydrate diet. This ratio approximates 0.60.

The results obtained with the high protein diet are presented in table 3. The daily thiamine requirement values which constitute the last column are not, as previously, "estimated to be within at least 2 μg. of the true value." In this instance they are the exact amounts of thiamine which prevented any significant rise in the urinary pyruvate excretion.

The 50% decrease in the amount of thiamine administered to a number of the rats during the first week attended the lowering of the food intake for a few days in order to stimulate the appetite. Rat 5 was destroyed and replaced by another normal control, rat 5 A, because it refused food and lost weight. The fact that its urine contained blood suggested some renal injury. A gross examination, however, revealed no obvious defect. The requirement values for rats 11 and 12 are missing because they also refused food; as a consequence of their loss of appetite, they were killed. Their pyruvate

[3] Crisco.

TABLE 3

High protein diet:

The relation of pyruvate excretion to the intake of thiamine.

PERIOD	1			2		3		4	
Rat No.	B_1/day		P.A.[1] in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.
	5 days	2 days		6 days		5 days		6 days	
	µg.	*µg.*	*mg.*	*µg.*	*mg.*	*µg.*	*mg.*	*µg.*	*mg.*
4	60 [2]	60 [3]	(3.1)[2]	55 [4]	3.8	55	3.7	55	3.5
5	60 [2]	60 [3]	(3.0)[2]	55 [4]	3.8	55 [3]	3.6	55 [3]	(2.9)[2]
6	60	60	3.5	55	3.4	55	3.5	55	3.4
7	18 [2]	9 [4]	(3.2)	18	4.0	18	4.3	18	4.6
8	20 [2]	10 [4]	(3.5)	20	3.7	20	4.1	20 [3]	4.0
9	22	22	3.9	22	3.5	22	4.0	22	4.0
10	19 [2]	10 [4]	3.5	19	3.5	19	4.1	19	3.8
11	19 [2]	10 [4]	3.4	19	3.2	19	3.5	19 [3]	(2.9)[2]
12	19 [2]	10 [4]	3.3	19	3.5	19	3.8	19	3.8
13	17	17	3.8	17	3.8	17	3.7	17	3.7
14	20 [2]	10 [4]	4.3	20	4.2	20	4.0	20	4.0
15	19	19	3.7	19	3.7	19	3.8	19	4.3
16	22	22	3.4	22	3.5	22	3.5	22	3.6
17	20 [2]	10 [4]	(3.2)[2]	20 [2]	3.5 [2]	20	3.5	20	3.8

PERIOD	5		6		7		DAILY B_1 REQUIREMENT
Rat No.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	B_1/day	P.A. in 24 hrs.	
	6 days		6 days		7 days		
	µg.	*mg.*	*µg.*	*mg.*	*µg.*	*mg.*	*µg.*
4	55	3.4	55 [3]	3.5	55 [2]	3.5 [2]	
5 A[5]	55 [4]	3.2	55	3.2	55	3.7	
6	55	3.4	55	3.5	55	3.6	
7	20	4.1	20	5.0	22	4.6	22
8	18	4.2	18	4.8	20	6.1	22 ?
9	20	4.3	20	4.0	18	4.6	20
10	17	4.4	17	4.6	19	4.8	19
11	Killed						
12	17	3.7	15	3.7 [2]	15 [3]	(4.1)[2]	..
13	15	4.0	15	4.5	15	4.4	17
14	18	4.2	18	4.4	18	4.1	18
15	19	4.4	21	4.4	21	4.3	21
16	20	3.6	20	3.5	18	3.5	18
17	18	4.1	18	4.1	20	3.7	20
					Average = 20		
					(Coefficient of variation = 9%)		

[1] Pyruvic acid (P.A.) determined on urine of last 24 hours in each period.

[2] Food refused during urine collection.

[3] Food refused at least once during period indicated; but not during urine collection, unless indicated.

[4] Food intake lowered to stimulate appetite.

[5] Rat 5 A, a control, substituted for rat 5 which refused food and lost weight.

values remained within normal range at all times so that a thiamine insufficiency was not the direct cause of their anorexia.

DISCUSSION

The deficient controls (rats 1–3) on the high carbohydrate diet reacted in a manner characteristic of a developing thiamine deficiency. They showed a fourfold increase in the excretion of urinary pyruvic acid in 3 weeks' time, although the final value for rat 2 was somehow unaccountably decreased. These regular increases offered some assurance that the deficient diet was, in fact, wholly devoid of, or at least very low in, thiamine.

The daily pyruvate excretions of the normal controls (high carbohydrate diet) were satisfactorily constant in spite of the fact that the collection represented only one 24-hour period out of 7 days. The excretion of rat 4 varied from 1.7 to 2.1 mg., that of rat 5 from 2.2 to 2.7 mg., and that of rat 6 from 2.4 to 3.1 mg. Some of the variation was due no doubt to the changes in body weight which approximately paralleled the pyruvate excretions: average initial weight, 327 gm., average initial pyruvate, 2.1 mg., average final weight, 385 gm., average final pyruvate, 2.4 mg.

The average daily thiamine requirement of 33 μg. (coef. var. = 8%) as given in table 2 is considerably above the value of 14 μg. which is obtained on the basis of Arnold and Elvehjem's ('39) suggestion that "the requirement for thiamine by most species of animals may be simply stated as approximately 1 microgram of the vitamin per gram of dietary solids." It is also higher than the value obtained by Mills ('41) who produced normal growth at 65°F. but not at 91°F. when he fed 0.6 to 0.8 μg. of thiamine per gram of diet as a supplement to the same diet that was used by Arnold and Elvehjem. Previously, Van Veen ('32) had found that young rats weighing less than 100 gm. required from 4 to 8 μg. of thiamine daily.

The disagreement is not wholly unexpected since, in the above investigations the rate of growth was used as a measure

of thiamine deficiency, whereas it is suggested by this investigation that the increase in urinary pyruvate serves as a much more delicate index. The animal organism apparently responds with an increase in pyruvate excretion before it shows any symptoms of anorexia. This is borne out by a consideration of the deficient controls. Rat 1 showed an increase in urinary pyruvate from a normal value of 1.8 μg. to a deficient value of 2.7 μg. in 1 week's time and refused food only on the seventh day. Rat 2 showed an increase from 1.8 to 6.7 μg. in 2 weeks' time and did not refuse food before the fourteenth day. Rat 3 almost tripled its pyruvate excretion in 14 days without exhibiting any signs of anorexia.

The pyruvate excretion of the normal controls was more constant on the high protein diet than it had been on the high carbohydrate diet. The excretion of rat 4 varied between 3.4 and 3.8 mg., and that of rat 6 between 3.4 and 3.6 mg. The first excretion value for rat 4 (3.1 mg.) is not included because the food intake was lowered during that collection period in order to stimulate the appetite.

The initial, basal pyruvate excretion of most of the animals was higher on the high protein diet than on the high carbohydrate diet. All of the normal controls and many of the assay animals responded with immediate increases when changed to the high protein diet. The remaining animals showed either a decrease or no change, depending on whether their final value when receiving the high carbohydrate diet had been high or only moderately high.

The average daily thiamine requirement of 20 μg. (coef. var. = 9%) is 39% lower than the 33 μg. (coef. var. = 8%) needed when a high carbohydrate diet is fed. On the strength of the data given above, that casein is approximately 42% glycogenic (50% corrected for nitrogen) and fat at least 10% glycogenic, the decrease was estimated at 50%. This change in the requirement is in agreement with the results of Funk, Collazo and Kaczmarek ('25). They found that pigeons were able to metabolize more protein than carbohydrate when given the same amount of vitamin B.

If, at this time, for purposes of approximation only, it is permissible to ignore the fat fractions of the two diets since they occur in but small and near-equal amounts, then it can be calculated, as shown below, that casein should not be more than 64% glycogenic. The equations:

$$8.96\,x + 2.69\,y = 33$$
$$2.16\,x + 6.75\,y = 20$$

yield values of 3.09 and 1.97 for x and y, respectively, where x and y are the micrograms of thiamine required per gram of sucrose and casein contained in the diets and their coefficients the grams of each of these substances that were fed each day (dry weight). The ratio 1.97:3.09 = 64:100. Although the total thiamine requirement values include the thiamine excreted in the urine and feces, any deductions for these factors would serve only to decrease the 64:100 ratio, since the excretion on the high protein diet must be equal to or less than that on the high carbohydrate diet; hence, the limitation, "not more than 64% glycogenic."

Under these circumstances a comparison of the two values, of which one (not more than 64% glycogenic) was obtained from experimental results, and the other (42% glycogenic) was calculated on the basis of data given by Schmidt ('38) and Hawk and Bergeim ('37), suggests that the glycogenic constituents of the diet are of major importance when studying thiamine requirement values. More precisely, it might be inferred that thiamine participates in the oxidation of the proteins to the extent of the glycogenic amino acids which they contain.

SUMMARY

The average daily thiamine requirement of ten, adult, male albino rats of the Wistar Institute strain weighing nearly 400 gm. and maintained in approximate energy equilibrium at 26°–30°C. on a diet containing 64% sucrose, 20% casein, and 10% hydrogenated cottonseed oil (plus cellulose flour, salts, and pure vitamin supplements) has been found to be 33 μg.

(coef. var. = 8%). On a diet high in protein (64% casein, 19.6% sucrose, 10% hydrogenated cottonseed oil; and representing a 9% decrease in the energy intake), nine of the ten rats had an average requirement of 20 μg. (coef. var. = 9%).

A comparison of the two values demonstrates a 39% reduction in the daily requirement.

The increase in the urinary pyruvate excretion (determined by the method of Lu) which indicates a possible disturbance in intermediary carbohydrate metabolism has been used as the index of subacute thiamine deficiency.

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THE RESPIRATORY QUOTIENT OF THE LIVER

I. DETERMINATION OF THE LIVER R.Q. IN ANESTHETIZED DOGS
II. INFLUENCE OF CERTAIN DIETS ON THE R.Q.

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With one exception (Blalock and Mason, '36), previous determinations of the respiratory quotient of the liver have been made on the artificially perfused organ or on tissue slices in the Warburg apparatus. In the perfusion technique (Blixenkrone-Møller, '38), no care was taken either to approximate the normal blood flow through the organ or to maintain normal levels of blood gas tension in the perfusion fluid. The respiratory quotients so determined usually showed a progressive decrease during the course of an experiment. The results obtained from tissue slices (Gemmill and Holmes, '35; Stadie, Zapp and Lukens, '40) possibly may not be representative of the total metabolic processes which occur normally. Those results obtained from the livers of normal, unanesthetized dogs (Blalock and Mason) vary over such a wide range that little confidence can be placed in a single determination.

It was with the view to developing a technique by which consistent determinations of the respiratory quotient of the liver could be made under better physiological conditions than heretofore that this study was undertaken.

[1] In partial fulfillment of the requirements for the degree of Doctor of Philosophy.

I. DETERMINATION OF THE LIVER R.Q. IN ANESTHETIZED DOGS

In order to estimate the R.Q. of the liver from a knowledge of the A-V gas differences and without knowing the total volume flow of blood, it is necessary to convert the double blood supply into a single one. Complete removal of one or the other source of inflowing blood is unsatisfactory since animals so treated show varying degrees of metabolic disturbances ranging from rapid death in the case of hepatic arterial ligation (Haberer, '06; Collens, Shelling and Byron, '27; Cameron and Mayes, '30) to less dramatic results in the case of the Eck fistula animal (Crandall and Roberts, '36; Smith and Whipple, '30). A single inlet may be attained, however, by anastomosing the common hepatic artery into the portal vein so that the liver receives both arterial and venous blood through one vessel.

This operation has been performed with some degree of success in the survival animal. Narath ('16) reported a few successful experiments in which the hepatic or renal artery was anastomosed into the portal vein. He concluded that such an anastomosis will serve to maintain the life of the animal and prevent the usual liver necrosis following complete exclusion of the normal hepatic arterial blood supply.

Rhoads and Chambers (unpublished observations) have attempted this operation in a number of dogs and succeeded in establishing chronically functional anastomoses in two animals which survived for more than a year, after which they were sacrificed. In all the other attempts, the dogs exhibited the usual pathological changes of the liver accompanying acute ligation of the hepatic artery, and were found to have completely thrombosed anastomoses. During the survival period in the two successful cases, no apparent clinical symptoms were manifested; blood sugar levels were normal and bromsulfalein tests showed no abnormality. It would appear from these results, that, if a successful anastomosis is attained, this operation will protect the animal against the harmful effects of hepatic artery ligation and will cause no gross changes in activity.

In view of the extreme technical difficulty of producing a chronically patent union between the hepatic artery and portal vein, it seemed advisable first to attempt the procedure in acute experiments, using a suitable anti-coagulant to maintain the anastomosis.

Dogs anesthetized with nembutal given intravenously were used. When required, the anesthesia was reinforced by intravenous barbital. Through a midline incision from xiphoid to umbilicus a clamp was placed on the common hepatic artery close to its origin from the celiac artery and a ligature tied about 3 cm. distal to this clamp; a metal cannula which pointed centrally was inserted between the clamp and ligature. The superior pancreatico-duodenal vein was ligated and a similar cannula, connected to the first by a short length of rubber tube, was inserted so that its opening projected well into the lumen of the portal vein. Chlorazol pink purified according to the method of Modell ('39) was injected intravenously, and the clamp on the hepatic artery released allowing arterial blood to pass into the portal vein. The hepatic artery proper was clamped just centrally to its first hepatic branch to prevent the very considerable collateral flow through the gastroduodenal artery which otherwise would occur.

In most of the experiments, finely-ground cinnabar was injected intra-arterially at the conclusion of the determination to check the absence of collateral circulation. Fine collaterals usually could be seen extending throughout the gastrohepatic omentum and surrounding the sheath of the gall bladder. No cinnabar was ever found in the blood in the deep tissue of the liver, and it is felt that the slight degree of collateral circulation described is insufficient to cause any detectable error in the determination.

A 1½ inch length of 18-gauge hypodermic needle, attached to a rubber tube, was inserted into the portal vein so that the point could be pushed above the entrance of arterial blood. A single suture served to hold the needle in place against the movements of the surrounding viscera. The socket of the needle was attached to the other end of the tube and led out

through the abdominal incision so that the manipulation of attaching a syringe would not tear the needle out of the vein.

A second 1½ inch length of 18-gauge hypodermic needle was bent in a flame to an approximate right angle and a rubber tube attached to the blunt end. Insertion of this needle, which is a rather delicate procedure, was made by gently retracting the liver caudally with one hand until a good exposure of the hepatic vein was secured. With the aid of a long hemostat the needle was inserted through the ventral wall of the vein, about 1 cm. cephalic to its junction with the liver tissue. The rubber tube was led out through the incision, taking care that it remained between the midline of the diaphragm and the liver as that organ was allowed to fall back into place, attached to the socket of the needle and fastened to the skin in the region of the xiphoid. No suture is necessary to hold the needle in the vein as the wall of that vessel is sufficiently thick to prevent its slipping out. When both needles are correctly in place there should be a spontaneous flow from the socket in the case of the portal vein and little resistance should be met when drawing blood into a syringe in the case of the hepatic vein. Except when drawing samples, the rubber tube is clamped tightly near the socket in each case.

A cannula was inserted in the trachea, and the blood pressure was recorded by a Hürthle manometer connected to a cannula in the carotid artery.

Samples of blood were obtained as follows: 1½ cc. was drawn from the portal vein, immediately followed by a similar volume from the hepatic vein. Each sample was transferred anaerobically to a mercury bulb and kept under positive pressure in an ice-bath until analyzed. This procedure was repeated at intervals of about 1½ minutes until twelve samples from each vessel had been collected and combined in the storage bulbs. As an additional precaution against clotting, approximately ½ cc. of 30% potassium oxalate was added to each reservoir. During the drawing of the blood samples, the expired air was collected in a Tissot spirometer connected to the tracheal cannula by a set of rubber flap valves previ-

ously tested for leaks. Inspired air was room air. The total time taken to make one complete collection of blood and expired air usually amounted to between 15 and 20 minutes.

A second determination as a check was made whenever possible about 15 minutes following the first, and after the blood which had been withdrawn had been replaced by citrated blood from a donor animal. Due to the unusually great loss of blood from the abdominal wound in the presence of the anti-coagulant, the amount of blood injected usually was two or three times that represented by the samples.

This method of drawing the samples differs somewhat from that used by Himwich and Castle ('27) in the case of muscle. These workers drew a single large sample simultaneously from the artery and vein, but it is the author's opinion that a large number of very small samples over a relatively long time yields more consistent results than a single large sample over a short period. An analogy lies in the usual method of collecting expired air for determination of oxygen consumption. An additional advantage of using the small-samples technique lies in circumventing the error caused when the blood is either accumulated in or drained from the organ. Edmunds ('15), Bauer, Dale, Poulsson and Richards ('32) and others have presented evidence that the liver may act as a temporary blood reservoir. Since a requirement for successful determination of the R.Q. of a single organ from the A-V gas differences is that the total inflow and outflow of blood be equal during sampling, storage of blood in the organ during the drawing of the samples could cause a serious error in the result; but if the sampling is prolonged, such an error will be avoided since no organ can change its volume in one direction indefinitely.

The oxygen and carbon dioxide contents of the blood were determined in duplicate by the Van Slyke manometric method (Van Slyke and Neill, '24). Oxygen and carbon dioxide capacities were determined by equilibrating 10 cc. portions of the blood with 5% carbon dioxide and 95% oxygen at room temperature and analyzing in the Van Slyke apparatus. R.Q.'s

were calculated after correcting the gas content for possible change in hemoglobin concentration in passing through the liver, according to the method of Himwich and Castle. Expired air samples were collected from the spirometer in Bailey bottles and kept under positive pressure until analyzed in duplicate for oxygen and carbon dioxide in the Haldane-Henderson apparatus (Henderson, '18). The R.Q.'s were calculated in the usual way. In a few instances total acetone bodies were determined by Van Slyke's method (Van Slyke and Fitz, '17) and the total fatty acid content of the liver by the method of Leathes and Raper ('25).

The success of the method may be judged by reference to the first column of tables 1 and 2. Duplicate determinations of the R.Q.'s (represented by two sets of figures in one experiment) agreed within ± 0.05 and in most cases within ± 0.03, except in experiments 11 and 32; the maximum range found was 0.24 in experiment 11. The greatest standard deviation for a series of animals on a given diet was ± 0.08. These values show a much greater degree of consistency than those obtained by Blalock and Mason on the livers of unanesthetized dogs, and by Himwich and Castle on resting muscle.

The cause of the exceptionally wide variation found in experiment 11 may have been due to the fact that the blood pressure had dropped to a level so low as to be indicative of a state of severe shock. In such a condition, carbohydrate may have been mobilized, and the high R.Q. simply reflects the participation of the liver tissue in the burning of this carbohydrate. The great variation in experiment 32 undoubtedly was due in part to the unusually low A-V gas differences, such low values serving to exaggerate the effect on the R.Q. of the analytical error of ± 0.2 volumes per cent.

II. INFLUENCE OF CERTAIN DIETS ON THE R.Q.

Previous to the experiment the animals were subjected to four different types of dietary regimes. The first type consisted of canned dog food given during at least a week previously; animals on this diet were in the post-absorptive state

(20 hours) before operation. The second type was starvation over a period of 2½ to 8 days. The third was a high fat diet in which approximately 60% of the daily caloric intake consisted of fat in the form of lard or corn oil, the remainder of the diet being canned dog food with added vitamins A, B_1, and D. The total caloric value of this ration approximated that of the first. In one case the diet consisted entirely of 100 gm. of corn oil daily, following 2 days of starvation. This was successful in only one instance, due to the weakness of the animal following a week or so of such treatment. The fourth type was identical to the high-fat diet with the exception of the addition of 2 gm. of choline chloride daily, in one case from the start of the regime and in another case for only the last 4 days. With the latter two diets, as with the first, the animals were in the post-absorptive state before operation.

In every case with the meat-fed, post-absorptive dog the value of the R.Q. fell within the physiological range. The average value of 0.80 is higher than most of those reported by Blixenkrone-Møller but it agrees fairly well with Blalock and Mason's average value of 0.74, and with Gemmill and Holmes' average value of 0.79 obtained on liver slices from rats fed normal diets.

There is no evidence in these results that, under normal conditions of diet, the majority of the metabolic processes of the liver of the dog are not carried to completion.

The average value of 0.59 obtained on the starvation diet is significantly lower than that of the post-absorptive, meat-fed animal. It may be of interest to point out that one of the determinations on this diet (0.61, experiment 15) was made while the hepatic artery proper was open. This experiment must be considered as technically in error due to the collateral pathway allowed through the open vessel, but it nevertheless seems to have had but slight influence on the determination.

Having no information other than the value of the R.Q., it is impossible to say anything positive concerning the cause of the low value in the case of this diet. R.Q.'s below 0.70 have frequently been cited as an indication of the trans-

TABLE 1

R.Q.'s of dogs on a diet of canned food and starvation previous to operation.

DIET	R.Q.		ARTERIAL-VENOUS DIFFERENCE		MINUTE VOLUME
	Liver	Exp. air	O_2	CO_2	
			vol.%	*vol.%*	*liter*
I — Canned dog food					
Exp. 8 — 2/19/41 Body wt. 13.9 kg.	0.76	....	14.3	10.9	....
Exp. 11 — 3/20/41	0.69	....	6.2	4.3	....
Body wt. 9.8 kg.	0.93 [1]	....	7.7	8.3	....
Exp. 12 — 3/27/41 Body wt. 9.1 kg.	0.78	....	9.1	7.1	....
Exp. 13 — 4/3/41 Body wt. 8.8 kg.	0.85	0.80	5.3	4.5	4.17
Exp. 20 — 6/9/41	0.81	0.85	9.3	7.5	5.19
Body wt. 14.9 kg.	0.77	0.87	11.8	9.1	Lost
Exp. 22 — 6/16/41 Body wt. 9.3 kg.	0.82	1.0	9.5	7.8	4.43
Mean	0.80				
Stand. deviation	±0.07				
Mean of those in which exp. air was determined	0.81	0.87			
II — Starvation					
Exp. 15 — 4/17/41	0.61 [2]	0.85 [2]	6.2	3.8	5.85
Body wt. 12.5 kg. 2.5 days	0.56	0.82	6.4	3.6	6.63
Exp. 25 — 7/10/41	0.66	0.86	7.3	4.8	3.36
Wt. 7.1 kg. — loss of 1.4 kg. — 6.5 days	0.57	0.77	6.7	3.8	3.69
Exp. 26 — 7/24/41	0.59	0.83	7.8	4.6	3.67
Wt. 9.3 kg. — loss of 1.2 kg. — 8 days	0.57	0.82	6.9	3.9	5.03
Mean	0.59				
Stand. deviation	±0.04				

[1] Animal in shock — blood pressure 59 mm. Hg.

[2] Not included in statistical analyses.

formation of fat into carbohydrate, a process which is said to have an R.Q. of less than 0.3 (Stadie, Zapp and Lukens, '41). In view of the evidence of Stadie, Zapp and Lukens ('40) and of Crandall, Ivy and Ehni ('40), however, it is more likely

TABLE 2

R.Q.'s of dogs on a high fat and high fat + choline diet previous to operation.

DIET	R.Q.		ARTERIAL-VENOUS DIFFERENCE		BLOOD KETONES AS ACETONE		LIVER FAT	MINUTE VOLUME
	Liver	Exp. air	O_2	CO_2	Portal	Hepatic		
			vol.%	*vol.%*	*mg.%*	*mg.%*	%	*liter*
III — High fat								
Exp. 24 [1] — 7/2/41								
2 days fast, 5 days fat	0.73	0.85	8.9	6.5	....	....	....	3.44
Wt. 6.7 kg. — loss 1.7 kg.	0.78	0.86	8.5	6.6	....	....	....	4.90
Exp. 29 — 11/25/41	0.53	....	3.0	1.6	14 on mixed bloods		....	....
22 days high fat								
Wt. 11.0 kg. — loss 1.6 kg.	0.49	....	4.5	2.2				
Exp. 31 — 1/9/42	0.58	0.84	2.4	1.4	3	2	2.8	....
39 days high fat	0.68	0.90	3.1	2.1				
Mean	0.57							
Stand. deviation	±0.08							
IV — Choline								
Exp. 30 — 12/2/41								
High fat + 2 gm. choline	0.82	....	9.9	8.1	1	2	9.7	....
Wt. 11.6 kg. — loss 1.0 kg.	0.85	....	12.8	10.9				
Exp. 32 — 1/16/42								
High fat 46 days 2 gm.	0.63	0.82	2.4	1.5	13	1	3.5	2.90
choline daily last 4 days.	0.47	0.86	3.4	1.6				3.13
Wt. 11.0 kg.								

[1] Not included in statistical analyses.

that the R.Q. of 0.59 represents the production of ketone bodies from fat by the liver. Such a process has an R.Q. of 0.65 according to the theory of the successive B-oxidation of the fat molecule, and zero according to the theory of multiple alternate oxidation. Thus, if the liver were putting out ketone bodies,

the expected value of the R.Q. of the organ might well fall below 0.70, or the value for the complete oxidation of the fat molecule.

The average value of 0.57, obtained on animals on the high fat diet in which 60% of the caloric intake was in the form of lard or corn oil, is significantly lower than that of the meat-fed dog but is not significantly different from that on the starvation diet. In experiment 29, the high value of ketones found in the blood lends support to the possibility that this low R.Q. is representative of the formation of ketones from fat by the liver. Unfortunately the analysis for liver fat was lost in this instance, but it is worthwhile mentioning that the liver of this animal had the yellowish color typical of livers with high fat content. This was the only instance where such a condition was so pronounced as to be worthy of note. In experiment 31 the results of the blood ketone and liver fat analyses do not indicate that the animal had anything but a normal level of fat in the liver and scarcely any ketone production. Duplicate analyses of acetone and fat agreed very well in this case, but the methods used may have yielded different results in more experienced hands. Quite possibly the R.Q. may be a much more sensitive indicator of the general course of fat metabolism in the liver than measurements of the ketone body production or fat level.

In the single instance where the diet consisted of 2 days of starvation followed by 5 days of pure fat feeding, the R.Q.'s were above 0.70. In view of the preceding results the reason for this is a complete mystery. Two attempts were made to obtain additional information on this point, but for reasons previously given further trials were abandoned. This result should be checked in the future.

In the single experiment performed with a diet of high fat supplemented with 2 gm. of choline chloride daily, the R.Q. of the liver was 0.84. The blood ketone analysis indicated no abnormal production of ketones by the liver, yet the liver fat was at the abnormally high level of 9.7%. On the basis of this single experiment, it cannot be said that choline

given in conjunction with a high fat diet will prevent ketone production from fat by the liver. It is felt that these results are of sufficient interest to warrant further intensive investigation of this subject; it is surprising that the liver fat was so high in this instance. In the face of other evidence (Best, Ferguson and Hershey, '33) on the effect of choline on the accumulation of fat in the liver, these results in experiment 30 cannot be satisfactorily explained. Possibly it may be due to individual variation in the response of the animal to the substance.

In experiment 32, choline was given during the last 4 days of the high fat regime with the idea of investigating the possibility of the transformation of fat into carbohydrate as a partial explanation of the disappearance of fat from the livers of animals fed high fat diets followed by choline. If such a process occurred to any appreciable degree, the R.Q. of livers so treated should fall to a significantly lower level than that obtained on the animal fed a high-fat ration without the later addition of choline. As can be seen from the results, no indication of any such transformation was obtained. The average R.Q. was not appreciably different from those obtained on the high-fat, no-choline diets. This may have been due, however, to the failure of the liver to accumulate fat during the 42 days preceding the choline feeding.

The results of the blood ketones analysis in the case of experiment 32 are peculiar in that considerably more ketones were found in the blood entering the liver than in that leaving. This is completely unexplainable on the basis of any present evidence.

Another point of importance is the comparison of the R.Q.'s of the liver with those of the entire animal as obtained from the expired air. The mean of the expired air quotients of animals on the canned food diet was not significantly different from that of the liver quotients on the same diet (considering only those experiments where both liver and expired air quotients were obtained). It would appear from these results that the total metabolic oxidations occurring in the liver are

similar to those occurring in the entire animal in the case of meat-fed, post-absorptive dogs.

In the case of the starvation diet, however, there is a very real difference between the R.Q. of the liver and of the entire body. This difference can easily be explained on the basis that the end-products of whatever unusual metabolic process occurred in the liver to lower the R.Q. were completely oxidized by the tissues of the rest of the body. There is no significant difference between the R.Q.'s of the expired air of the meat-fed and starved animals. Crandall, Ivy and Ehni concluded, on the basis of urinary output, that 95% of the ketones produced by the livers of their starved and fat-fed animals was oxidized by other tissues of the body.

It is possible that some of the expired air R.Q.'s are higher than would be expected in a meat-fed or starved dog. The value of 1.0 in experiment 32 is undoubtedly too high. The minute volumes are, with the exception of experiment 32, somewhat higher than would be expected in a dog. This could, of course, account for the high R.Q. due to "blowing-off" of carbon dioxide. During the course of the majority of the experiments it was observed that the animals tended to exhibit a rather rapid, panting type of respiration, and frequently it was observed that the carbon dioxide capacity of the portal blood fell slightly between consecutive determinations. Even if it was assumed that the true expired air R.Q.'s were on a somewhat lower level the general relationship between the liver and expired air quotients would not be affected qualitatively.

CONCLUSIONS

1. A method has been developed by which consistent determinations of the R.Q. of the liver in vivo can be made.

2. The R.Q. of the liver was found to have a value greater than 0.70 and to be not significantly different from the R.Q. of the entire animal when previously fed a meat diet.

3. The R.Q. of the liver was found to be lower than 0.70 in animals previously starved or fed a high fat diet.

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SENSORY NEURON DEGENERATION IN PIGS

IV. PROTECTION AFFORDED BY CALCIUM PANTOTHENATE AND PYRIDOXINE [1, 2]

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SIX FIGURES

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In previous reports (Wintrobe et al., '38, '40, '42), abnormal gait and degenerative changes in the nervous system of pigs have been described. It was pointed out that these lesions occurred as the result of a dietary lack of some substance present in whole liver and, less consistently, in brewers' yeast. In past experiments in this series it was shown that this disorder developed in animals fed diets containing adequate amounts of thiamine, riboflavin, nicotinic acid, wheat germ oil, and cod liver oil, as well as adequate protein, carbohydrate, fat and minerals.

In an attempt to discover the nature of the deficiency which leads to the development of the changes in the nervous system,

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[2] These observations were presented in abstract form at the 29th Annual Meeting of the American Society for Experimental Pathology in Boston, Massachusetts, April 3, 1942.

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experiments were planned with the object of producing deficiencies of single vitamins. This appeared possible when pyridoxine and pantothenic acid became available in sufficient quantities, for it then was found in preliminary observations that good growth occurred and excellent health was maintained in pigs fed our basal diet together with these two vitamins in addition to thiamine, riboflavin, nicotinic acid, choline and cod liver oil.

The present report describes the effects produced when, of these vitamins, the following were *not* furnished in crystalline form in concurrent experiments with comparable groups of animals: group (1) thiamine; (2) riboflavin; (3) nicotinic acid; (4) pyridoxine; (5) choline; (6) calcium pantothenate; and (7) vitamin A. In several groups two or more vitamins were not furnished, as follows: group (8) pyridoxine and choline; (9) choline and calcium pantothenate; and (10) pyridoxine, choline, and calcium pantothenate. There were two control groups; namely, group (11) in which none of these vitamins was omitted, and group (11 a) in which none of these vitamins was omitted but highly purified ("vitamin free") casein was fed instead of the "crude" casein given the remainder of the animals. In a final group (12) desiccated whole liver was furnished instead of the above six "B" vitamins.

It is to be noted that with the exception of group (11 a) the animals received in their basal diet a grade of casein which cannot be regarded as vitamin free. According to our assays, made by the microbiological technique (Strong et al., '41), this casein contained approximately 400 µg. riboflavin per 100 gm. In view of the observations to be described it is possible that it furnished some nicotinic acid and vitamin A, although we have no information as to the actual amounts of these two vitamins which may be carried in the casein. Evidence will be cited which indicates that it furnishes insufficient nicotinic acid to permit good growth under certain conditions. Whatever the content of nicotinic acid, vitamin A and other vitamins such as biotin and inositol in our basal

diet may be, it is clear from the observations to be described that little thiamine, pyridoxine or pantothenic acid was available to the pigs other than as given in crystalline form.

A review of the literature was presented in a previous report (Wintrobe, Miller and Lisco, '40).

GENERAL PROCEDURE

Included in this report are observations on two large lots of animals, those numbered from 6–29 to 6–50, inclusive (experiment VI) having been begun on the experiment in October, 1940, and those numbered from 6–51 to 6–80, inclusive (experiment VII) having been started in March, 1941.

The animals were received at our laboratory shortly after weaning at an age of approximately 3 weeks. The feeding, housing, and observation of the pigs were similar to that described elsewhere (Wintrobe, Miller and Lisco, '40). From the time of their arrival until the termination of the experiment their basal diet had the following percentage composition: "New Process" casein [6], 26.1; sucrose, 57.7; lard, 11.0; and swine salt mixture no. 3 (Wintrobe, Miller and Lisco, '40), 5.2.

During the first 4–5 months of each of the experiments each animal was fed 36.4 gm. of this diet per kilogram body weight per day. After the fifth month this was gradually reduced to one-half. The reduction in caloric intake was associated with no appreciable reduction in weight gain.

The basal diet was supplemented with cod liver oil [7] (0.5 gm. per kilogram body weight daily) except in group 7, which received viosterol [7] diluted in corn oil in amounts furnishing 87.5 international units vitamin D per kilogram body weight.

All animals were initially fed 3 gm. of brewers' yeast [7] per kilogram body weight per day. In experiment VI (animals numbered from 6–29 to 6–50, inclusive), a progressive reduction in the yeast supplement was begun when the pigs were 90–96 days of age, the yeast being entirely omitted when the animals were 104–110 days old. Vitamin supplementation as

[6] Sheffield By-Products Co.

[7] Mead Johnson and Company.

indicated in table 1 was begun at 97 to 103 days of age. In experiment VII (pigs 6–51 to 6–80, inclusive), the reduction of yeast was begun when the pigs were 73–75 days old and its omission was completed when they were 87–89 days of age. In this experiment the vitamin supplementation was begun at 80–82 days of age. In experiment VII the animals were placed on wire screens from the age of 5 months in an effort to prevent coprophagy.

TABLE 1

Nature and quantity of vitamin supplements fed each experimental group.

SUPPLEMENT [1]	GROUP NUMBER													AMOUNT FED KG./BODY WEIGHT/DAY
	1	2	3	4	5	6	7	8	9	10	11	11a[2]	12[3]	
Thiamine hydrochloride		+	+	+	+	+	+	+	+	+	+	+		0.52 mg.[4]
Riboflavin	+		+	+	+	+	+	+	+	+	+	+		0.12 mg.
Nicotinic acid	+	+		+	+	+	+	+	+	+	+	+		1.20 mg.
Pyridoxine hydrochloride	+	+	+		+	+	+		+		+	+		0.20 mg.
Choline chloride	+	+	+	+		+	+				+	+		10.00 mg.
Calcium pantothenate	+	+	+	+	+		+	+			+	+		0.50 mg.
Vitamin A	+	+	+	+	+	+		+	+	+	+	+	+	900 I.U.
Vitamin D	+	+	+	+	+	+	+	+	+	+	+	+	+	87.5 I.U.

[1] The vitamins were fed by capsule every other day. The actual amount given was revised every 2 weeks according to the most recently recorded weight of each animal. In giving the vitamins, the capsule was placed far back in the animal's oropharynx. The pig was then observed for 5 or 10 minutes, and, if regurgitation of the capsule occurred, a second one was given.

[2] Group 11a was given acid, alkali and alcohol-washed casein instead of the less refined product fed the rest of the animals.

[3] Group 12 was given desiccated whole liver, 1.5 gm. per kilogram body weight daily, in the place of crystalline vitamins.

[4] This dosage was reduced to 0.13 mg. per kilogram per day in pigs numbered 6-51 to 6-80, inclusive, at 130–140 days of age.

The preliminary feeding of yeast was carried out in order to ensure satisfactory weaning and to allow the more gradual development of vitamin deficiency than would result if the animals were placed on the experimental regime at once.

All the animals were autopsied and blocks of all the tissues placed in formalin. Microscopic examinations were made of all the organs except the brain and eyes from one animal in each of the thirteen groups. If pathological alterations were noted in any of these, similar tissues from the other animals

in the group were examined. Because of our special interest in the nervous system, the brachial and sciatic nerves, spinal ganglia and spinal cord of every animal were examined. The brain and eyes were saved for study.

OBSERVATIONS

Details of the general observations do not come within the scope of this report but a few need mention.

Growth. Growth curves for all the animals appear in figure 1. It is obvious that none of our animals quite equalled the rate of growth of comparable pigs on a well-balanced diet of crude foodstuffs. Good growth was achieved, however, in those pigs receiving six "B" vitamins in addition to cod liver oil (groups 11 and 11 a), and in the animals receiving no crystalline nicotinic acid (group 3), no vitamin A (group 7), no added choline (group 5) and no crystalline riboflavin (group 2). Pantothenic acid deficiency, whether alone (group 6) or in combination with a lack of choline (group 9) or with pyridoxine and choline deficiency (group 10), produced the most marked disturbance of growth. Pyridoxine deficiency also reduced the rate of growth (groups 4 and 8) but not to the degree produced by pantothenic acid deficiency.

Appearance of animals. Malnutrition in the pig is usually manifested first by an appearance of untidiness which is in marked contrast with the well-groomed, clean-looking healthy pig. In pigs given no pyridoxine (group 4) or calcium pantothenate (group 6) and in those deficient in both of these vitamins (groups 8, 9 and 10) the coats often appeared thin and dry. In those deficient in pantothenic acid the alopecia was sometimes quite marked and was accompanied by some reddening of the skin. Thinning of the hair was most noticeable over the rumps and along the spinal column. Failure to supplement the basal diet with choline (group 5) seemed to bear little relation to the appearance of the animals and those given no added riboflavin (group 2) or nicotinic acid (group 3) appeared in excellent condition throughout the experiment. The omission of vitamin A from the supplement

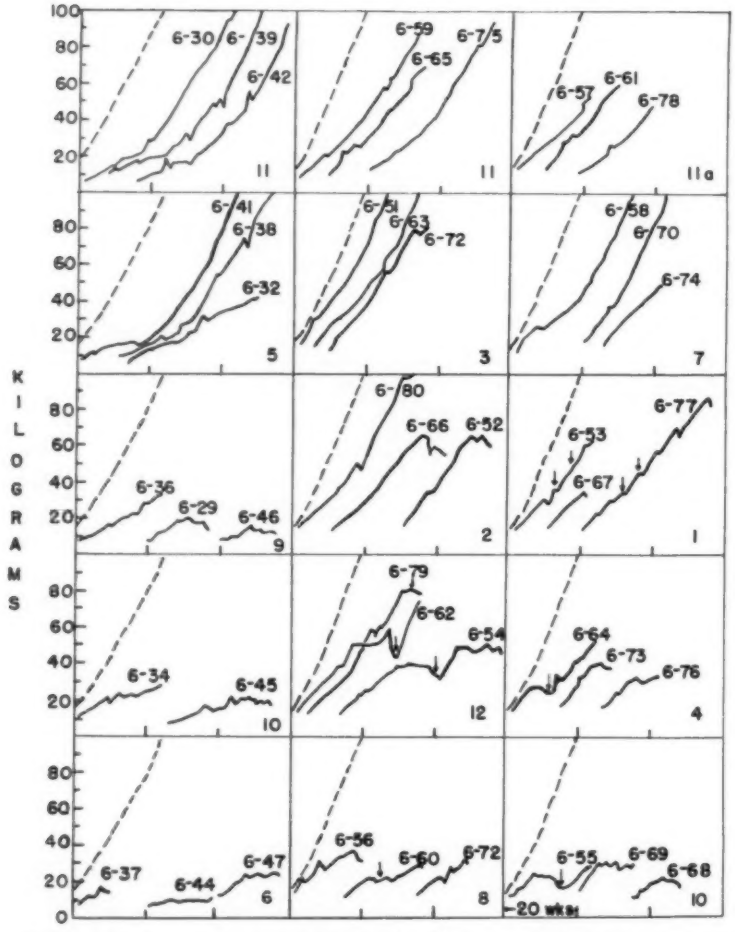

Fig. 1 Growth curves of animals in the different experimental groups as described in table 1. All curves begin at the time when crystalline vitamin supplements were added to the basal diet. The broken line represents the growth of pigs given a mixed diet at the Beltsville Research Center and fed and handled according to record of performance procedure.

(group 7) was not associated with any changes in the hair or skin of the affected animals.

Alimentary system. Loss of appetite and vomiting were the first signs of deficiency to appear in the pigs which received no thiamine (group 1), and were quickly succeeded by other more serious signs. As in the thiamine-deficient pigs vomiting was encountered in all the animals given desiccated liver (group 12) instead of synthetic vitamins. This symptom was usually preceded or accompanied by anorexia. In both these groups these two signs could not be correlated with any pathological changes. That they were closely related to changes in the blood pyruvic acid level, and were the result of thiamine deficiency is supported by the fact that the appetite returned and vomiting ceased when thiamine in sufficient dosage was administered. These and other observations in animals deficient in thiamine will be reported separately (Wintrobe, Stein, Miller, Follis, Najjar, and Humphreys, '42; Follis, Miller, Wintrobe and Stein, '42).

Anorexia also occurred in animals receiving neither pyridoxine (groups 4 and 8), calcium pantothenate (groups 6 and 9), nor either vitamin (group 10), but in these pigs it appeared as a late phenomenon of deficiency and was only rarely accompanied by vomiting.

Glossitis was not observed in any of the animals. Ulceration was found irregularly in the stomach but its incidence seemed more related to that of trichobezoars than to any form of nutritional deficiency.

Diarrhea occurred with frequency whenever the vitamin supplement failed to include calcium pantothenate (groups 6, 9 and 10) or, less regularly, when pyridoxine was lacking (groups 4, 8 and 10). Only in uncomplicated pantothenic acid deficiency (group 6), were changes in the bowel noted. The pathological change was confined to the colon, the process consisting of widespread, patchy injection of the mucosal surface of the entire large bowel. Microscopically there was necrosis of the superficial portion of the mucosa with leuko-

cytic infiltration of the remaining mucous membrane and submucosa. Numerous glands were dilated and their lumens filled with leukocytes (fig. 2).

Nervous system. Abnormal gait and sensory neuron degeneration (figs. 3, 4 and 5). The abnormal gait that occurred in these experiments was of the same type as that previously observed (Wintrobe et al., '40, '42). The affected animal at first shows only a slightly high lift of the hind legs in walking

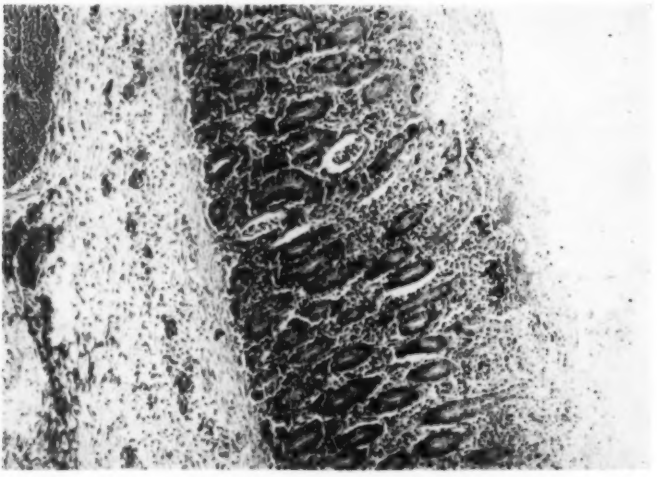

Fig. 2 Photomicrograph of mucosa of colon of a pig deficient in pantothenic acid (6-37) showing necrosis of the superficial layer of the mucosa with leukocytic infiltration of the remainder. There are perivascular accumulations of cells about the blood vessels in the submucosa. × 90.

and there is some swaying of the hind quarters. As the condition progresses a definite "goose step" develops, and the animal stumbles and falls repeatedly. When ataxia has become marked, the hind legs assume grotesque positions even when the pig is at rest. In only a few animals has the abnormality involved the forelegs and then only long after the characteristic gait had affected the hind quarters.

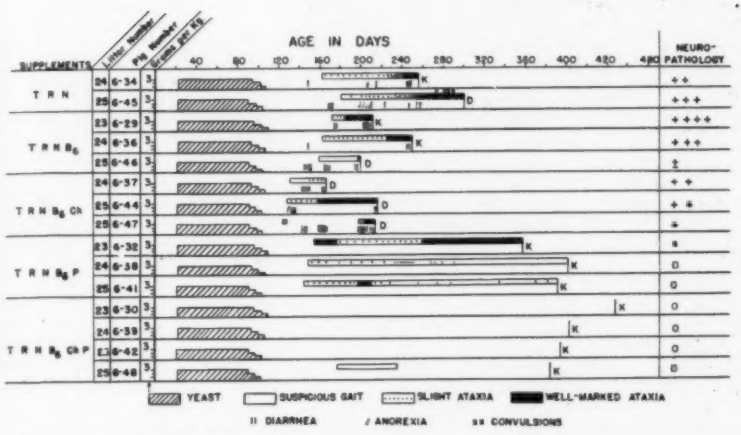

Fig. 3 Experiment VI. The development of abnormal gait, convulsions, loss of appetite and diarrhea, and the extent of neuropathological changes in pigs given a diet low in water-soluble vitamins other than as supplied in the form of the pure, synthetic vitamins indicated. All the pigs received the same basal diet. Vitamin supplements were started when the quantity of yeast was reduced below 3 gm. per kilogram and were continued throughout the life of the pigs.

Note the freedom from any symptoms of deficiency whatsoever in the pigs given thiamine (T), riboflavin (R), nicotinic acid (N), pyridoxine (B_6), choline (Ch) and calcium pantothenate (P), and the development of abnormal gait and neuropathological changes when pantothenic acid or pantothenic acid as well as pyridoxine was lacking.

Convulsions were observed only in the animals not given pyridoxine.

Abnormal gait, as described in the text, was observed in all the pigs given no choline, but neuropathological changes were found only in one animal. These were of a type not seen heretofore and are therefore marked *.

D indicates that the animal died, K that it was sacrificed.

The scoring of neuropathological changes is that described in the footnote to table 2.

Abnormal gait as well as sensory neuron degeneration occurred in all pigs failing to receive either pyridoxine (group 4) or calcium pantothenate (group 6), alone or in certain combinations involving also a lack of choline (pyridoxine and choline, group 8; calcium pantothenate and choline, group 9; pyridoxine, calcium pantothenate and choline, group 10). Abnormal gait was not observed in pig 6–71 of group 8, an animal which died after repeated convulsions had taken place during

TABLE 2

Data concerning incidence of abnormal gait, treatment and pathological changes in all animals.

GROUP NO. AND VITAMIN SUPPLEMENT [1]	PIG NO. AND SEX	AGE SUPPLEMENT STARTED	AGE ATAXIA OBSERVED	AGE AND MANNER OF DEATH [2]	PATHOLOGICAL CHANGES	
					Nervous system [4]	Other tissues
		days	*days*			
1 RNB_6ChP (no. T)	6-53 M	87 [3]	.....	246 D	0	Focal necroses, myocardium Hemorrhage and edema, lung
	6-67 F	87	.....	171 D	0	Focal necroses, myocardium Edema, lung
	6-77 F	87 [3]	.....	325 D	0	Focal necroses and scars, myocardium Edema, lung
2 TNB_6ChP (no R)	6-52 M	87	.....	260 K	0	Ulcer, tongue; abscesses, l. hind leg and between vertebrae. Adhesions, pericardium and pleurae
	6-66 F	87	.....	312 K	0	Abscesses, both hind legs
	6-80 F	87	.....	314 K	0	.....
3 TRB_6ChP (no N)	6-51 M	87	.....	289 K	0	Healed ulcer, stomach
	6-63 M	88	.....	292 K	0	.....
	6-72 F	88	.....	285 K	0	Small abscesses, kidney
4 TRNChP (no B_6)	6-64 M [5]	87	137 [3]	259 K	+	Ulcer, stomach Slight hemosiderosis, spleen
	6-73 F [5]	87	152	197 D	+ [3]	Autolysis; hemosiderosis, spleen, liver and marrow; fatty liver, mod.
	6-76 F [5]	88	141	202 K	++	Fatty liver, mod.; hemosiderosis, spleen, liver and marrow
5 $TRNB_6P$ (no Ch)	6-32 F	110	270	357 K	Lat. [6]	Fatty liver, marked
	6-38 F	107	232(270) [7]	402 K	0	Fatty liver, marked; ulcer, stomach; adhesions, pleura
	6-41 M	106	198(228) [7]	390 K	0	Fatty liver, marked
6 $TRNB_6Ch$ (no P)	6-37 M	107	142(?) [8]	166 D	++	Colitis
	6-44 M	104	145	217 D	++	Colitis
	6-47 F	104	201	214 D	+++	Colitis
7 $TRNB_6ChP$ (no A)	6-58 F	87	302	342 K	0	Healed fractures, ribs
	6-70 M	87	290	315 K	0	Focal necroses, voluntary muscle
	6-74 F	87	.....	205 D	0	.....
8 TRNP (no. B_6Ch)	6-56 F [5]	88	205	229 K	+	Fatty liver, mod.; hemosiderosis, spleen, liver and marrow
	6-60 M [5]	87	149 [3]	242 K	++++	Fatty liver, sl.; hemorrhage, stomach Hemosiderosis, spleen, liver and marrow Gen. caseous lymphadenopathy
	6-71 M [5]	87	.....	187 D	±	Fatty liver, marked; hemorrhage, stomach; subarachnoid hemorrhage; Hemosiderosis, spleen, liver and mar.
9 $TRNB_6$ (no ChP)	6-29 F	110	183	211 K	++++	Abscesses, skin
	6-36 M	107	224	240 K	+++	Fatty liver, slight
	6-46 M	106	199	200 D	±	Lobular pneumonia

TABLE 2 — (*Continued*)

GROUP NO. AND VITAMIN SUPPLEMENT [1]	PIG NO. AND SEX	AGE SUPPLEMENT STARTED	AGE ATAXIA OBSERVED	AGE AND MANNER OF DEATH [2]	PATHOLOGICAL CHANGES	
					Nervous system [4]	Other tissues
		days	*days*			
10 TRN (no B_6ChP)	6-34 M	107	238	257 K	++	Hematomata, both hams; hemosiderosis, spleen, liver and b. m. Fatty liver, extreme
	6-45 M [5]	104	255	300 D	+++	Fatty liver, moderate Hemosiderosis, spleen and marrow
	6-55 F [5]	87	158 [3]	256 K	+++	Pleural and pericardial adhesions Fatty liver, moderate
	6-68 F	87	151 [3]	189 D	+++	Fibrinous pericarditis; hemosiderosis, spleen and marrow; fatty liver, sl.
	6-69 M	87	135 [3]	198 D	+++	Lobular pneumonia; hemosiderosis, spleen and marrow; fibrinous pleurisy; fatty liver, moderate
11 TRNB_6ChP	6-30 F	106	.....	448 K	0	Postpartum uterus
	6-39 F	106	.....	402 K	0	Pregnant, 7 fetuses Ulcer, stomach
	6-42 M	106	.....	403 K	0	Abscesses, skin
	6-59 F	87	.....	328 K	0	.....
	6-65 M	88	.....	282 D	0	Acute gastritis
	6-75 F	87	.....	328 K	0	.....
	6-48 F	91	.....	320 K	0	.....
11a TRNB_6ChP (Vit.-free casein)	6-57 F	88	.....	236 K	0	.....
	6-61 M	87	.....	236 K	0	.....
	6-78 F	87	.....	238 K	0	.....
12 Des. liver (low T)	6-54 F	87 [3]	.....	410 D	0	Focal necroses, myocardium Edema, lung
	6-62 M	88 [3]	.....	327 K	0	.....
	6-79 F	87 [3]	.....	319 K	0	Healed fracture, vertebra

[1] T = thiamine, R = riboflavin, N = nicotinic acid, B_6 = pyridoxine, Ch = choline, P = calcium pantothenate. For purposes of ready reference the supplement not furnished is shown in brackets.

[2] Pigs 6-53, 6-77, 6-54, 6-62 and 6-79 were treated with thiamine from time to time. Treatment with pyridoxine, as described in the text, was commenced in the following pigs at the age shown in parentheses: 6-64 (157); 6-60 (174); 6-55 (187); 6-68 (181); 6-69 (185).

[3] K = killed; D = died.

[4] 0 = no lesions; + = definite changes in the peripheral nerves only: these consisted in myelin degeneration with free fat granules and swollen neurilemmal cells (when there was less extensive change in the peripheral nerves with only Marchi degeneration demonstrable, the lesion was graded as ±); ++ = changes were accompanied by chromatolysis in the cells of the posterior root ganglia; +++ = degeneration of the posterior roots and the root entry zone of the spinal cord, in addition to the ganglion cell and peripheral nerve changes already noted; ++++ = conspicuous degenerative changes in the nerves, ganglia, roots and posterior funiculi of the spinal cord.

[5] Tissues badly autolysed; cord and ganglion cell changes could not be studied.

[6] Degenerative changes in the lateral funiculi of the spinal cord and nowhere else.

[7] Pigs 6-38 and 6-41 were no longer ataxic after the day indicated in parentheses.

[8] Gait appeared mildly ataxic only on the 142nd day.

[9] Epileptiform convulsions were observed in the following animals, commencing on the days indicated in parentheses: 6-64 (157); 6-73 (157); 6-76 (165); 6-56 (203); 6-60 (154); 6-71 (158); 6-45 (273); 6-55 (168). Three of these were treated with pyridoxine and the convulsions did not reappear.

his third month on the experiment. At autopsy, however, even in this pig there were found early degenerative changes in the sciatic nerve in addition to a small subarachnoid hemorrhage. The latter probably developed following a convulsion and may have caused the animal's death.

Slightly abnormal gait was also seen in all of the animals given no supplement of choline (group 5), but was persistent in only one of the three (pig 6–32). In the other two members of this group the gait became normal again later in the experiment (see fig. 3 and table 2). The ataxia in these animals differed somewhat from that which we have seen previously inasmuch as the usual "goose step" and "spasticity" of the

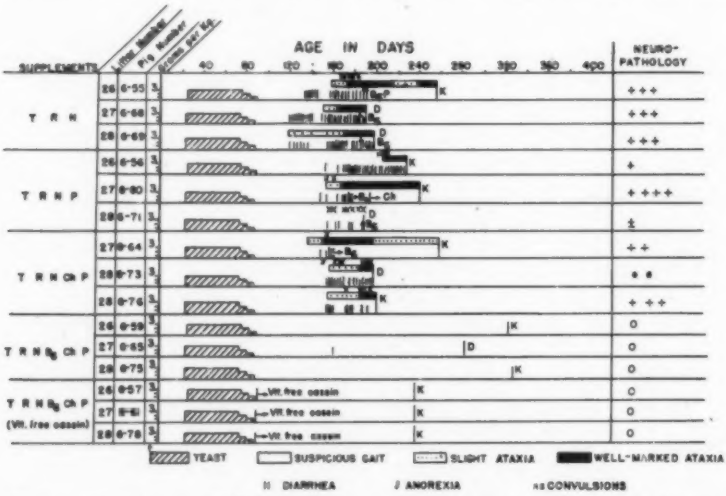

Fig. 4 Experiment VII. Legends same as in figure 3. Vitamin supplements were started when yeast was stopped.

Note the freedom from any symptoms of deficiency in pigs given thiamine, riboflavin, nicotinic acid, pyridoxine, choline and calcium pantotheanate, even when "vitamin-free" casein was fed instead of "crude" casein.

Abnormal gait and neuropathological changes developed when either pyridoxine or pyridoxine as well as pantothenic acid was lacking.

Convulsions were observed only in the animals not given pyridoxine.

Arrows pointing vertically represent single injections of pyridoxine (B_6) or calcium pantothenate (P), as indicated. Arrows pointing horizontally refer to daily administration.

hind legs were not observed. The gait had an unbalanced, staggering quality. Changes in the nervous system were seen only in pig 6–32. These consisted of myelin degeneration without gliosis and were confined to the lateral funiculi of the spinal cord. No alterations in the ganglia, peripheral nerves, or other spinal tracts were seen.

Two of the pigs in group 7 receiving no supplement of vitamin A, developed abnormal gaits quite late in the experiment. The animals exhibited chiefly a peculiar staggering manner of locomotion. In none of these animals were neuropathological changes noted, but foci of necrosis in the striated muscle, very similar to those described in experimental vitamin E deficiency in guinea pigs and rabbits (Mackenzie, Levine and McCollum, '40) were seen in one animal (6-70). These lesions were of only rare occurrence, however, and their significance is doubtful.

Neither abnormalities of gait nor histologic changes in the nervous system were observed in the animals given no crystalline supplement of either thiamine (group 1), riboflavin (group 2) or nicotinic acid (group 3).

Effect of treatment. Only five animals were treated after signs of deficiency had become apparent. Two of these succumbed within a few days after treatment was instituted (6-68, 6-69). Pig 6-55, which, like 6-68 and 6-69, had received neither pyridoxine, choline, nor calcium pantothenate, was given pyridoxine and calcium pantothenate in the usual maintenance dose (table 1) after it had become practically helpless from progressive ataxia of a month's duration. During the time that ataxia developed, anemia and inactivity had appeared and growth ceased. The improvement in the blood picture following treatment was dramatic. Growth, activity and appetite were resumed within 3 weeks. The gait improved considerably but more slowly and, when this animal was killed after 2 months of continuous treatment, the gait was still definitely but only mildly ataxic. Pig 6-60, in group 8, was given 110 mg. pyridoxine intravenously after ataxia had become progressively worse and had been present for at least

25 days. Treatment was continued orally with a daily dose of 200 μg. per kilogram body weight. Although the anemia which had concurrently developed disappeared rapidly, and convulsions ceased, the gait not only failed to improve but became worse. The remaining animal which received treatment (6-64) was given 10 mg. pyridoxine intravenously, following which 200 μg. per kilogram body weight was given orally each day. Ataxia had been present for 20 days and had progressed to a severe stage by the time therapy was begun.

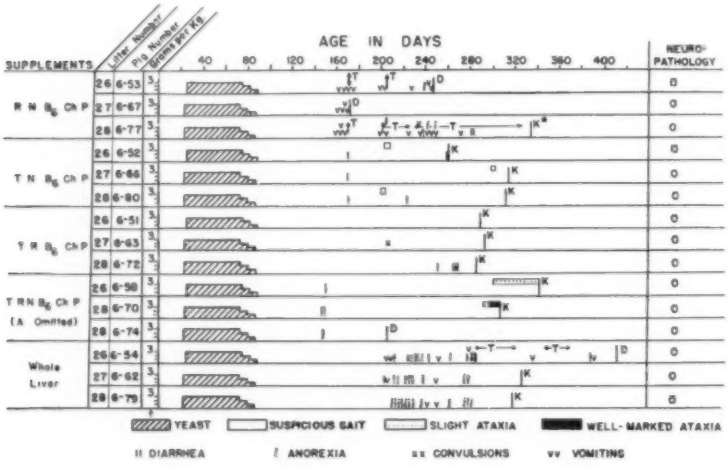

Fig. 5 Experiment VII, continued. Legends same as in figures 3 and 4.

Note the lack of neuropathological changes in animals given no crystalline thiamine, no riboflavin or no nicotinic acid. Pigs 6-58, 6-70 and 6-74 were given all the water soluble vitamins indicated but received no vitamin A. Although the gait became abnormal in two of these pigs, no neuropathological changes were observed.

Symptoms of nutritional deficiency, in the form of anorexia and vomiting were observed only in the pigs not given thiamine and in those fed desiccated whole liver, which contains little thiamine. Failure to develop signs of riboflavin deficiency was probably due to the content of this vitamin in the casein included in the basal diet. It is not known whether a similar explanation can be given for the failure to develop signs of nicotinic acid deficiency in the pigs given no crystalline nicotinic acid, and for the equivocal results in the animals given no supplement of vitamin A.

There was marked though gradual improvement in the gait over a period of 2 to 2½ months. As in the other treated animals, convulsions and anemia disappeared promptly and growth was resumed.

This number of observations is too small to allow conclusions to be drawn in regard to the effects of therapy. Further studies are in progress.

Convulsions. Epileptiform fits (Wintrobe et al., '42) occurred in various pigs which did not receive pyridoxine. These will be discussed in a separate report (Wintrobe, Miller, Follis and Stein, '42).

Anemia. Anemia occurred in all the animals whose supplement did not include pyridoxine. Marked hemosiderosis of the spleen, and to a lesser extent of the liver and bone marrow, was found in the pyridoxine deficient animals which were not treated. Details concerning the anemia and its response to treatment with this vitamin will appear in another report (Wintrobe, Follis, Miller, Stein, Humphreys and Suksta, '42).

Incidence of infection. Localized ulcerating infections occurred on the extremities of several pigs. These seemed to follow minor trauma, such as cuts and abrasions. Many fibrous adhesions were found in the pleural, pericardial and peritoneal cavities of those pigs which did not receive either pyridoxine, choline, or calcium pantothenate in experiment VII (see group 10, table 2). This was probably the result of some healed infection rather than the specific effect of deficiency, since such changes were not observed in comparable animals in experiment VI. Similar changes were noted in the pleura and pericardium of pigs 6-52 (group 2) and 6-38 (group 5).

Liver. An increased amount of fat in the liver (fig. 6) was found in animals given no supplement of either choline (group 5), pyridoxine or choline (group 8), or pyridoxine, choline or calcium pantothenate (group 10). However, a slight amount of fat was found in only one animal (6-36) of the group failing to receive either choline or calcium pantothenate (group 9). It is noteworthy, also, that fatty infiltration was

seen in pigs given choline but not pyridoxine (group 4), with the exception of the one animal (6-64) of this group which had been treated with pyridoxine. It would thus appear that fatty infiltration of the liver may arise when the diet lacks either choline, or pyridoxine, or both. When pantothenic acid was lacking in addition to choline, however, little or no fatty infiltration was found.

Fig. 6 Photomicrograph of liver of a pig not given choline (6-38), showing extremely fatty infiltration of the central and mid-zonal portions of the lobules. There is no cirrhosis, the connective tissue shown being normal for the pig. × 45.

Adrenals. In animals not given calcium pantothenate as a supplement (groups 6, 9 and 10) neither gross nor microscopic changes were found in the adrenals.

Gonads. Varying degrees of degeneration of the seminiferous tubules were observed in all animals in which poor growth occurred. On the other hand, gestation occurred in two of the pigs fed all six crystalline vitamins. In one of these animals (6-39) seven fetuses were unexpectedly found in the uterus at autopsy, while the second (6-30) gave birth to two pigs. One was found dead and the second died several hours after birth.

DISCUSSION

In table 3 are summarized the results of our studies involving the use of crystalline vitamins, as described in the present report and in earlier ones (Wintrobe et al., '38, '40), as well as those dealing with crude substances (Wintrobe et al., '42). It can be seen that sensory neuron degeneration developed in forty-one pigs when either pyridoxine or pantothenic acid, or both, were lacking; conversely, we have not observed such changes in the nervous system in any of twenty-six animals when both of these vitamins were furnished.

The basal diet, which included crude casein, sugar, lard, a salt mixture and cod liver oil, did not in itself furnish protective substances since all of the animals mentioned above as developing neurological changes were fed the same basal diet. Furthermore, the possible presence in crude casein of protective factors is ruled out by the fact that neurological changes did not occur when a purified casein was substituted for crude casein in the diets of animals receiving pyridoxine and calcium pantothenate in addition to thiamine, riboflavin, nicotinic acid and choline.

The conclusion that pyridoxine and pantothenic acid are necessary in maintaining the integrity of the nervous system in the pig is consistent with our earlier observations concerning the role of crude substances (table 3). Whole liver is an excellent source of both of these substances and different brands of yeast contain 30 to 75 μg. of pyridoxine per gram and 30 to 200 μg. of pantothenic acid.

In table 4 is given the amount of pantothenic acid in various fractions of liver, together with a roughly graded comparison of the potency of each fraction in preventing sensory neuron degeneration as described by us recently (Wintrobe et al., '42). The figures for pantothenic acid were obtained by the microbiological technique (Pennington, Snell and Williams, '40). An unsuccessful attempt was made to determine the pyridoxine content of these fractions by a chemical method (Scudi, '41).

TABLE 3

Summary of observations on nutritional deficiencies in pigs.[9]

SUPPLEMENT GIVEN [*]	NO. OF PIGS	GROWTH	ANOREXIA OR VOMITING	DIARRHEA	HAIR UNTIDY OR THIN	CONVULSIONS	ANEMIA	NEUROPATHOLOGY				
								0	+	++	+++	++++
T	3	±	+	+++	++++	0	++	–	–	2	1	–
TR	3	±	+	+++	++++	0	+	–	–	1	–	2
TN	3	+	+	++	+++	0	++	–	–	2	1	–
TRN [1]	20	+	+	++	++	+	++	–	–	9	6	5
$TRNB_6$	3	+	+	++	++	0	0	–	1	–	1	1
TRNP	3	+	+	++	++	+++	+++	–	2	–	–	1
$TRNB_6Ch$	3	+	+	+++	++	0	0	–	–	2	1	–
TRNChP	3	+	+	+	+	++	+++	–	1 [2]	1	1	–
$TRNB_6P$	3	++	0	0	0	0	0	2	1 [3]	–	–	–
TRB_6ChP	3	++++	0	0	0	0	+ [4]	3	–	–	–	–
TNB_6ChP	3	+++	0	0	0	0	+ [5]	3	–	–	–	–
RNB_6ChP	3	++	+++	0	0	0	0	3	–	–	–	–
$TRNB_6ChP$ [5]	17	+++	0	0	0	0	0	17	–	–	–	–
Yeast, type N	6	++	0	+	0	0	0	3	–	1	1	1
Yeast, types N + F	3	++	0	+	0	0	0	–	2	–	1	–
Yeast, types N + M	3	++	0	+	0	0	0	2	–	–	1	–
Yeast, type M [6]	5	+++	0	0	0	0	0	5	–	–	–	–
Whole liver	3	++	+++	0	0	0	+	3	–	–	–	–
Whole liver + TRN [7]	12	+++	0	0	0	0	0	12	–	–	–	–
"Press cake" [8] + TRN	3	++	0	0	+	++	++	–	2	1	–	–
"Whipple" fraction + TRN	3	+	+	+	+++	0	+	–	–	3	–	–
"Cohn" fraction + TRN	1	++	0	0	0	+	++	–	1	–	–	–
"Parenteral" fraction + TRN	3	++	0	0	0	+	++	1	2	–	–	–
Permutit fraction + TRN	3	+	+	++	++++	0	+	–	–	2	1	–
Mixture + TRN	3	+++	0	0	0	0	0	3	–	–	–	–
	118											

[1] Of these animals three were given wheat germ oil in addition to TRN.

[2] Tissues badly autolyzed; cord and ganglion cell changes could not be studied. Changes in the nerves were as severe as those in the other two pigs; therefore grading should probably be ++ or +++.

[3] Degenerative changes only in the lateral spinal funiculi.

[4] Anemia in these two groups was mild and related to infection.

[5] Three of these animals were not given vitamin A.

[6] Of these animals two were given wheat germ oil in addition to type "M" yeast. Wheat germ oil has been shown (Wintrobe, Miller and Lisco, '40) to afford no protection against sensory neuron degeneration.

[7] Of these animals, three were given wheat germ oil and type "M" yeast in addition to desiccated liver.

[8] This and the succeeding fractions of liver were given orally and are described fully elsewhere (Wintrobe et al., '42).

[9] It is to be noted that the chief object of these experiments has been to produce chronic deficiencies. Except in three animals described in the text, the basal diet included relatively crude casein rather than the "vitamin free" type. Furthermore, the supplement of yeast given all the pigs in their earlier weeks of life was usually removed only gradually as the supplement to be assayed was added to the diet.

[*] For meaning of various letters see footnote 1 to table 2.

It will be noted that the relatively high content of pantothenic acid in the "parenteral" and "Cohn" fractions of liver is consistent with the finding that these were superior to other fractions in preventing sensory neuron degeneration but were not as effective as whole liver.

Three brands of yeast were used in our earlier studies. Unfortunately assays for the content of pyridoxine and pantothenic acid in all three of these at the time they were used are not available. It is noteworthy, however, that all animals

TABLE 4

Content of pantothenic acid in liver and liver fractions, with a rough comparison of their potency in protecting the pig from sensory neuron degeneration.

	PANTOTHENIC ACID [1]	DEGREE OF PROTECTION [2]
	µg.	
Desiccated whole liver	213	Complete
"Press cake"	45	Poor
"Whipple" fraction	8.8	Very poor
"Cohn" fraction	76	Fair
"Parenteral" liver extract	82	Good
"Permutit" fraction	1.3	Very poor
Mixture of "press cake," "Whipple," "parenteral," and "permutit" fractions	137	Complete

[1] The figures represent the amount of vitamin found in that quantity of each substance derived from 5 gm. fresh liver.

[2] In grading the "degree of protection," the quantity of each fraction fed, the duration of time prior to the onset of ataxia, and the degree of neuropathology have been considered.

given type "M" yeast (see table 3) were protected and it seems plausible to assume that this yeast was superior quantitatively and perhaps qualitatively to type "N." "F" yeast was never used continuously in any group of animals.

Our observations in pigs give no support to the view that thiamine deficiency results in damage to the nervous system. Definite thiamine deficiency was produced in this experiment. Anorexia, vomiting and elevation in blood pyruvic acid occurred, as well as myocardial changes similar to but more extensive than those recently described in pigeons (Swank,

'40) and in dogs (Swank, Porter and Yeomans, '41) as resulting from thiamine deficiency. Although a deficiency state of 84 to 238 days' duration was produced, neurological lesions were not found in either the peripheral nerves, spinal ganglia or spinal cord of any of our animals.

The absence of all signs of nutritional deficiency in the pigs given no crystalline riboflavin (group 2) and in those given no nicotinic acid (group 3), as well as the equivocal results in those given no supplement of either choline (group 5) or vitamin A (group 7) makes it seem possible that these substances were supplied in sufficient amounts in the basal diet, particularly in the casein. According to assays of the casein, the pigs received as much as 40 μg. riboflavin per kilogram body weight through this medium. We have no adequate data regarding the content of the other above-mentioned vitamins in the basal diet. Consequently the results in respect to them must be regarded as inconclusive. In regard to choline it should be pointed out that the basal diet contained a high proportion of casein, thus making the conditions for the production of choline deficiency unfavorable.

In respect to nicotinic acid it should be pointed out that in earlier experiments in which the same type of casein was used as in the present series, when nicotinic acid was added to the same basal diet supplemented only with thiamine or with thiamine and riboflavin, striking improvement in rate of growth and in the condition of the pigs was observed (Wintrobe, Miller and Lisco, '40). Clearly the basal diet did not then furnish optimal amounts of nicotinic acid. Unless the content of this vitamin in casein varies from lot to lot, it would appear that the needs for nicotinic acid are different when only thiamine and riboflavin are furnished in crystalline form as compared with the requirements when pyridoxine, choline and calcium pantothenate are given as well.

SUMMARY

1. Young pigs fed a ration of crude casein, lard, sucrose and a salt mixture, supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine, choline, calcium pantothenate and cod

liver oil developed normally and exhibited no changes in the nervous system during life or at autopsy.

2. Pigs similarly fed but receiving supplements of B vitamins which failed to include either calcium pantothenate or pyridoxine, developed an abnormal gait and showed degenerative changes in the peripheral nerves, the posterior root ganglia, the posterior roots, and the posterior funiculi of the spinal cord.

3. In the animals whose supplements did not contain calcium pantothenate, a subacute inflammation of the colon was found, in addition to the changes in the nervous system.

4. In the animals whose supplements did not contain pyridoxine, epileptiform convulsions and anemia were observed in addition to the above changes in the nervous system. Both the convulsions and the anemia disappeared promptly following the administration of pyridoxine.

5. When thiamine was not furnished with the vitamin supplements no changes in the nervous system were observed although other signs attributable to thiamine deficiency developed.

6. The omission of choline was associated with some abnormality of gait in all of three animals but lesions were observed in the nervous system of only one. These lesions, furthermore, differed from those observed in association with pyridoxine or pantothenic acid deficiency.

7. The results of observations on 118 pigs are summarized and the signs of chronic deficiency of various water soluble vitamins are presented.

8. It is concluded that both pyridoxine and pantothenic acid are necessary in maintaining the integrity of the nervous system in the pig under the conditions of these experiments.

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CALCIUM, PHOSPHORUS AND NITROGEN METABOLISM OF YOUNG COLLEGE WOMEN [1]

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THREE FIGURES

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Although the years of late adolescence and early adulthood of women are important from the standpoint of nutrition, little is known concerning the nutrients ingested or the retention of such nutrients by young women in this age group. Even a casual observation of the food eaten by a group of individuals shows wide differences in food habits; differences which are largely due to custom, which in turn has been influenced by many factors, including the economic one. The controlled diets of balance experiments are duplicated to a very small extent by the population at large. Outhouse et al. ('41) showed that calcium needs, computed for individuals on fixed diets, could apply only to individuals who were on similar diets.

Information as to the amounts of nutrients ingested and retained when women are eating their customary diets should

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undoubtedly be considered in any discussion of recommended food allowances. A few studies have been made on intakes and retentions of women on their self-chosen diets (Wang, Hawks, Huddlestun, Wood and Smith, '30; Hetler, '32; Coons and Schiefelbusch, '32; Hunscher, Donelson, Erickson and Macy, '34).

As part of a long-time study of the nutritional status of college women as related to their dietary habits (Nelson, '38), a study of calcium, phosphorus and nitrogen intakes and retentions or losses was made of a large group of young college women during the years of 1936 to 1941. The results of these studies which are here reported are from three sources: Part I, which dealt with a group of college women observed during one or more 7- or 10-day periods during which the women ate their customary self-chosen diets; Part II, which dealt with one woman studied for forty-four 5-day periods while she was on her usual diet; and Part III, which dealt with a group of nine women studied for three periods of 10 days each while the women were eating basal diets supplemented by definite amounts of milk. Most of the observations were made between menstrual periods.

The subjects were all presumably in good health, carrying on their usual curricular and extracurricular activities, and continued their usual manner of life during the period of the study. Living and eating arrangements of the women considered in Parts I and II were representative of living arrangements of young women enrolled in land-grant colleges. The group included young women who had their meals at home; at sorority houses; at university houses, where the women prepared their own meals; at university eating places; and at university residence halls. Whatever the living arrangement, some choices as to foods eaten were inevitable, and foods eaten during the time each girl's balance was studied represented her customary diet.

Of a total of 109 individuals included in Part I, fourteen were observed at intervals during 2 or more years. For example, one girl's balance was studied when she was 19, again

when she was 20, and then again when she was 21, age being calculated to the nearest birthday. In order to study any effect which age might have on retention, it was decided to include each of these subjects in each of the age groups in which she was at the time of the observation. Considering an individual in each age group in which she was at the time of observation, the total number of observations are reported as 124 rather than 109. Of the 124 observations, eighty-four were made in Nebraska, twenty-eight in Ohio, nine in Kansas, and three in Iowa.

Daily records of foods with amounts eaten were kept for every subject during each observation period. Inspection of the records showed that the majority of the subjects used some foods from each of the following groups during each period: milk and milk products; potatoes and sweet potatoes; tomatoes and citrus fruits; leafy, green, and yellow vegetables; other fruits and vegetables; eggs; lean meat, poultry or fish; flour and cereals; fat including butter; and sugar. Amounts of the different foods used varied and the quality of each subject's diet depended upon the amounts used from each food group. Many of the diets were undoubtedly above the average in quality.

Because it was found early in the study that A. H. in Iowa, the individual included in Part II, failed to retain calcium on an intake of less than 1 gm., her daily food intake was planned to allow 4 cups of milk. Otherwise her diet was unrestricted.

During the school years 1939–1940 and 1940–1941, a study was made of the calcium, phosphorus, and nitrogen intakes and retentions of nine young women when they were on basal diets supplemented during each of three periods by 1, 2, and 3 cups of milk, respectively. The basal diets used were adaptations of diets used in a metabolism study by Leverton and Roberts ('37). Foods included in the basal diets during each period were the same in kind and amount for each individual and were such that, with the exception of calcium, the diets as a whole were adequate. The amounts of calcium in the diets

varied with the amounts of milk used. Certain foods of negligible mineral content, such as white bread, butter, jelly, sugar, and candy, were used ad libitum to meet the varying calorie needs of the individuals.

These young women lived in their usual places of residence. Their meals, planned to include the basal diet plus the designated amounts of milk, were prepared and served to them.

This study was divided into a 10-day collection period at each level of milk intake, each collection period being preceded by a 15-day foreperiod. Studies of four of the nine subjects on the controlled diets were made in Ohio, three in Iowa, and two in Kansas.

METHODS

The customary procedure used in collecting food samples and excreta was used. Food was weighed as eaten and sampled, and samples were pooled into a composite representing an aliquot of each day's intake for each individual. At the end of the period each composite sample was digested with concentrated HCl, sieved, made up to a convenient volume, thoroughly mixed and stored for analysis at a later date. Total feces for the individual for each period were collected, carmine being used as a marker, and the composite sample was made into an acid digest and stored. Urine was collected daily, aliquots were acidified with HCl, and the composite of the aliquots for each subject was analyzed, without preliminary ashing. Aliquots of the acid digests of food and feces were ashed for calcium and phosphorus determinations according to the method of Stearns ('29). For calcium determinations a modification of the McCrudden method as described by Stearns ('29) was used; for phosphorus, the Fiske and Subbarow method ('25); and for the nitrogen, the Kjeldahl procedure, (Association of Official Agricultural Chemists, '30). Similar procedures in collection and preparation of samples and in chemical analysis were followed in each of the four states.

RESULTS AND DISCUSSION

For the 124 women included in Part I of the study, certain decisions to be made concerning the validity of using the data so collected necessitated statistical analysis. The method of variance and covariance as described by Snedecor ('40) was used as an aid in making these decisions as indicated in the following paragraphs.

Intra-individual differences

In some cases a young woman's balance was studied for two or more periods during 1 year. Variations from period to period of such individuals were significantly less than variations from individual to individual. As shown by the mean squares of retention adjusted for intake, the intra-individual variance was 0.011 as compared to an inter-individual variance of 0.024. Accordingly the averages for individuals observed more than once during a year have been reported.

Place differences

Mean intakes and retentions differed from state to state. Analysis of the data showed that variance in retentions could be reduced by adjusting to the mean intake. The difference between the retentions adjusted for intake of the Nebraska and the Ohio groups was found not to be significant, as indicated by the use of Student's t-test. The number of cases in the other two groups were too small to justify analysis or to have much influence on the figures for the entire group. Consequently state differences have been disregarded in the study of the group of 124 individuals.

Age differences

Jeans and Stearns ('39) quote Todd as stating that mineralization of the skeleton does not always keep pace with the rate of skeletal growth. For some time after growth ceases, one might expect a continued deposition of skeletal minerals, and this deposition would be reflected in the greater mineral

retention during such periods if adequate intakes were the rule. The exact age at which complete mineralization occurs probably varies widely among individuals. Analysis of the data by age groups for these young college women, however, showed mean calcium retentions which did not differ significantly among the various age groups. Possibly physiological and chronological ages may have varied to such an extent in each age group that the two were confused. Certainly no indication as to when storage ceased was shown by the data. A detail analysis, using age in years and fractions thereof, showed no relation between balance and age for the one subject, A.H. Age differences have therefore also been disregarded in the results reported.

Weight differences

An analysis, by means of multiple regression of calcium intake and body weight upon calcium retention, gave a correlation coefficient, $R = 0.504$, which was only slightly greater than the correlation coefficient of calcium intake upon calcium retention, $r = 0.502$. In addition, calcium intake was not significantly related to weight as shown by an r of 0.078. Weight differences have therefore been disregarded in the results presented.

The data for the 124 women as reported therefore, include mean daily figures for each individual even though some individuals were studied more than once during a year, and disregard place, age, and weight differences.

Calcium intakes

The mean daily calcium intake, 0.941 gm. (table 1) for the large group of 124 women was similar to the daily mean intakes of 0.93 and 0.95 gm. reported by Coons and Schiefelbusch ('32) and Hunscher et al. ('34), respectively, and came within the range of what Sherman and Lanford ('41) refer to as a desirable population standard of from 0.9 to 1 gm. per capita daily. The mean daily intake for the large Nebraska

TABLE 1

Calcium, phosphorus and nitrogen metabolism data according to intake levels for 195 observations (in grams).

DAILY INTAKE RANGE	CUSTOMARY SELF-CHOSEN DIETS								CONTROLLED DIETS			
	Part I. 124 individuals with 124 studies				Part II. One individual (A.H.) with 44 studies				Part III. Nine individuals with 27 studies			
	No. of studies	Mean intake	Mean balance	Percentage Individuals Retaining	No. of studies	Mean intake	Mean balance	Percentage Individuals Retaining	No. of studies	Mean intake	Mean balance	Percentage Individuals Retaining
Calcium												
0.322–.399	1	0.322	—.089	0	..	....	...	..	..	....	...	...
0.400–.499	6	0.469	—.139	17	..	....	...	..	..	....	...	...
0.500–.599	11	0.561	—.025	36	..	....	...	..	3	0.560	.019	100
0.600–.699	14	0.659	—.033	43	..	....	...	..	5	0.648	.035	60
0.700–.799	12	0.753	—.040	50	..	....	...	..	2	0.765	.028	50
0.800–.899	16	0.850	.034	62	..	....	...	..	3	0.809	—.061	0
0.900–.999	13	0.939	—.019	46	7	0.934	—.110	0	2	0.940	.067	100
1.000–1.099	18	1.045	.044	72	5	1.058	.042	80	3	1.061	.133	100
1.100–1.199	12	1.145	.111	83	2	1.108	.074	100	5	1.147	.104	100
1.200–1.299	4	1.261	.125	100	13	1.255	.043	62	3	1.264	.076	100
1.300–1.399	6	1.344	.142	83	9	1.359	.180	89	1	1.398	.100	100
1.400–1.499	4	1.464	.227	100	6	1.429	.108	83	..	....	...	...
1.500–1.599	4	1.523	.234	75	1	1.552	.362	100	..	....	...	...
1.600–2.323	3	1.885	.180	100	1	1.653	.309	100	..	....	...	...
Means		0.941	.030			1.236	.072			0.921	.055	
Phosphorus												
0.663–.699	3	0.675	—.136	0	..	....	...	..	..	....	...	...
0.700–.799	10	0.762	—.140	10	..	....	...	..	..	....	...	...
0.800–.899	11	0.853	—.049	36	..	....	...	..	..	....	...	...
0.900–.999	7	0.951	.062	86	1	0.990	—.177	0	4	0.956	.100	100
1.000–1.099	20	1.052	—.042	40	5	1.026	.016	60	2	1.060	.146	100
1.100–1.199	21	1.145	.109	90	7	1.151	.145	86	7	1.141	.002	57
1.200–1.299	18	1.246	.103	89	15	1.242	.168	93	2	1.277	.116	100
1.300–1.399	7	1.351	.175	100	9	1.357	.181	89	6	1.355	.054	50
1.400–1.499	9	1.432	.144	89	5	1.424	.302	100	2	1.441	.161	100
1.500–1.599	7	1.544	.161	100	2	1.542	.290	100	2	1.592	.291	100
1.600–1.699	3	1.668	.197	100	..	....	...	..	2	1.673	.284	100
1.700–1.799	4	1.742	.331	100	..	....	...	..	..	....	...	...
1.800–2.125	4	1.901	.185	100	..	....	...	..	..	....	...	...
Means		1.177	.062			1.255	.164			1.260	.101	
Nitrogen												
5.55–5.99	1	5.55	—1.67	0	..	....	...	..	..	....	...	...
6.00–6.99	3	6.70	—0.72	33	..	....	...	..	..	....	...	...
7.00–7.99	14	7.51	—0.62	14	..	....	...	..	..	....	...	...
8.00–8.99	19	8.51	0.02	63	4	8.78	0.26	50	2	8.85	0.70	100
9.00–9.99	26	9.52	0.38	69	17	9.60	1.22	100	2	9.06	0.19	50
10.00–10.99	26	10.45	1.09	92	15	10.43	1.71	100	7	10.45	0.64	86
11.00–11.99	15	11.47	1.07	80	6	11.11	2.79	100	6	11.36	0.86	83
12.00–12.99	11	12.38	1.37	100	2	12.28	3.05	100	7	12.27	1.36	86
13.00–13.99	3	13.41	1.53	100	..	....	...	..	3	13.51	1.20	100
14.00–14.99	3	14.65	2.59	100	..	....	...	..	..	....	...	...
15.00–17.58	3	16.27	2.89	100	..	....	...	..	..	....	...	...
Means		10.10	0.63			10.13	1.59			11.24	0.91	

group (0.856 gm.) was practically the same as the slightly lower daily allowance for women recommended by the Committee on Food and Nutrition of the National Research Council ('41).

Variations in intake among individual subjects were large and ranged from a low of 0.322 gm. (B.B. in Ohio) to a high of 2.323 gm. (R.H. in Kansas), but observation of foods used by groups of individuals shows that calcium intakes do vary widely and that the variation as shown by this group of young women probably presents a typical picture of the custom of the general population of young college women in regard to calcium intake (fig. 1).

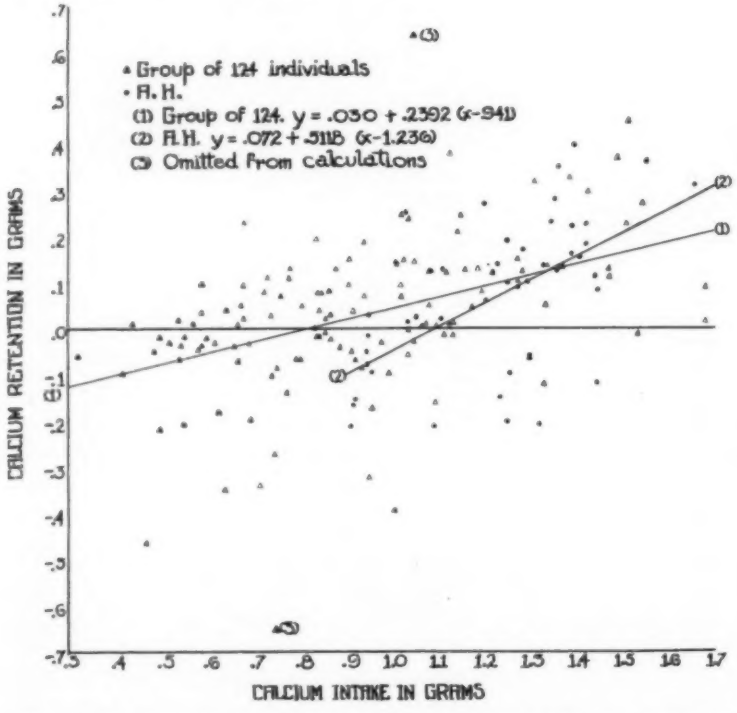
▲ Group of 124 individuals
• R.H.
(1) Group of 124. y = .030 + .2392 (x-.941)
(2) R.H. y = .072 + .5118 (x-1.236)
(3) Omitted from calculations
CALCIUM RETENTION IN GRAMS
CALCIUM INTAKE IN GRAMS

Fig. 1 The regression of calcium retention on calcium intake. One individual, intake 2.323 gm. and retention 0.217 gm., was omitted from the graph, but the figures were included in the calculation of the regression coefficient.

As shown in table 1, forty-four women or 35% of the 124, had less than the recommended 0.8 gm. daily.

The individual, A.H., for whom results are considered as of Part II, had a generous calcium intake, which ranged from a daily mean of 0.910 to 1.653 gm. with a mean of 1.236 for the entire forty-four studies. This amount exceeded the mean of the group of 124 individuals by 0.295 gm.

Calcium intakes of the nine women considered in Part III varied with the level of milk used, mean daily figures for the group being 0.629, 0.921, and 1.214 gm. as 1, 2 or 3 cups of milk, respectively, were used in addition to the basal diet. At the 1-cup level of milk ingestion the calcium intakes were all less than the recommended 0.8 gm. daily.

Calcium retentions

For the 124 women, retention was significantly related to intake as shown by a coefficient of correlation of 0.50. Losses were more frequent than were retentions at mean intake levels of less than 0.8 gm., with the percentage of individuals who were storing calcium at these low levels increasing from 0 at an intake of 0.3 gm. to 50 at an intake of 0.7 to 0.8 gm. (table 1). At intake levels of 0.8 gm. and above, mean retentions were evident at all but one level (0.900–0.999), with the percentage of individuals storing calcium increasing fairly consistently as the intake level increased.

Mean daily retentions varied among individuals on similar intakes. For example, twelve individuals having mean daily intakes ranging from 1.006 to 1.048 had balances ranging from —0.394 to 0.250 (fig. 1).

It is a commonly-accepted fact that even on a controlled diet an individual's retention may vary from period to period; and that different individuals show varying responses to similar intakes. Steggerda and Mitchell ('41) and Breiter et al. ('41) report extreme variability of calcium retentions for adults on the same types of diet. The variations in retentions found among these individuals on similar calcium

intakes in which the diets varied in other factors are therefore not surprising.

Reasons for differences in response are obscure. It has been assumed that well-nourished individuals utilize calcium less efficiently than those whose skeletal tissues have been depleted, but Breiter et al. ('41) found that of seven subjects observed, the three who showed the highest utilization of calcium were well-nourished in regard to this element.

The regression of balance on intake (fig. 1) shows the rate at which retention may be expected to increase when intake is increased as calculated from the data obtained for the 124 women. The regression line intersects the line of equilibrium at the point 0.816 gm. The indication therefore is that the young women in this group of 124 required an intake of that amount (0.816 gm.) to maintain themselves in equilibrium while they were eating their customary diets.

For A.H. (Part II), also, retention was significantly dependent upon intake, as shown by a coefficient of correlation of 0.56.

No retentions were evident at an intake level of less than 1 gm. Average figures showed retention at each intake level of 1 gm. and over, but the percentage of retentions at each level fluctuated to a greater extent than was the case for the group reported in Part I.

The regression of balance on intake for A.H. (fig. 1) shows that although her retention increased with increased intake the regression line intersects the line of equilibrium at 1.095 as compared to 0.816 for the large group.

When the mean daily calcium intakes of the nine women (Part III) ranged from 0.5 to 0.8 gm. the mean daily retentions were 0.019, 0.035, and 0.028 (table 1) for the three intake levels represented. Corresponding figures for the group of 124 (Part I) were —0.025, —0.033, and —0.040. The differences between the response of the two groups at the three levels were 0.044, 0.068 and 0.068 gm., respectively. Although these differences were small, they made storage possible. In addition, 70% of the group on the controlled diets

at these levels stored calcium at intake levels of less than 0.8 gm. as compared to 43% of the group who were eating their customary diets.

At intake levels above 0.8 gm., differences between retentions of the women on the controlled and those on their customary diets were less pronounced, the mean daily retention of the former being 0.070 gm. as compared to a corresponding figure of 0.055 gm. for the latter. Eighty-two per cent of the women on controlled diets stored calcium, as compared to 70% of those on their usual diets.

Undoubtedly the amount of calcium in the self-chosen diets was directly related to the milk intakes. Increased amounts of milk improve the quality of the diet in other factors as well as in calcium. At the lower levels of calcium intake, a decidedly larger proportion of the group having the controlled diets were in equilibrium or were storing calcium than was the case with the group who were having their customary self-chosen diets. It seems logical to assume that the smaller amounts of calcium were therefore more efficiently utilized when the diet was so selected as to provide adequately for other dietary factors than was the case when the women were on their customary diets.

Phosphorus intakes

The mean daily phosphorus intake for the group of 124 (1.177 gm.) (table 1) was similar to the 1.19 gm. reported by Coons and Schiefelbusch ('32), lower than the 1.523 gm. reported by Hunscher et al. ('34) and lower than the commonly-accepted Sherman standard of 1.32 gm. Inspection of table 1 shows that although the range in mean daily phosphorus intakes was large (0.663–2.125) the intakes clustered around the mean to a larger extent than was the case in regard to calcium. This was shown by a coefficient of variation of 25% for phosphorus as compared to a coefficient of variation of 34% for calcium.

Mean phosphorus intake for A.H. (Part II) was 1.255 with a range of from 0.990 to 1.574 and a coefficient of variation

of 11%, as compared to a coefficient of variation of 15% for calcium intake. Her mean intake for the forty-four periods of study (1.255) was only slightly below the Sherman standard.

Mean daily intakes of the group of nine women on controlled diets increased as the level of milk increased, mean daily intake figures for the group being 1.044, 1.245 and 1.492 gm. as 1, 2 or 3 cups of milk were used in addition to the basal diet.

Phosphorus retentions

For the group of 124 women, the phosphorus retentions were significantly dependent upon intake, as shown by a coefficient of correlation of 0.58. Losses were more frequent than retentions at intake levels of less than 1.1 gm. At levels of 1.1 and above, 93% of the individuals were retaining phosphorus. Figure 2 shows the regression of balance on

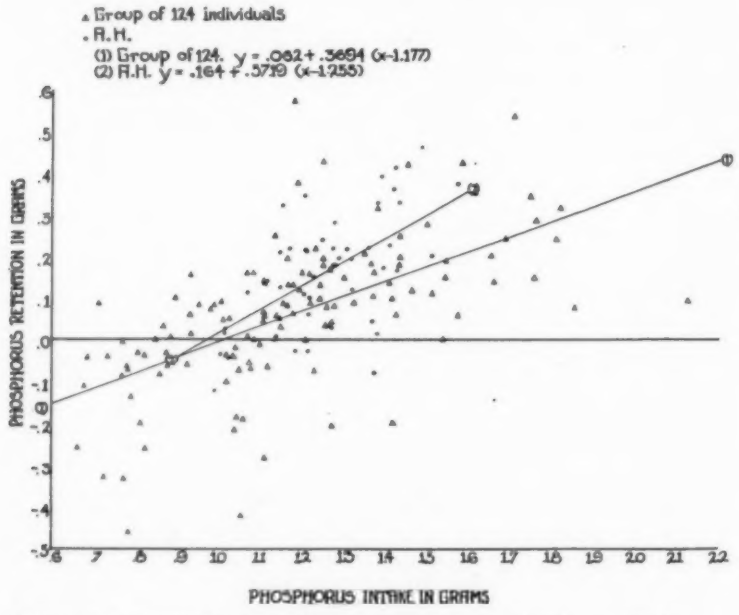
▲ Group of 124 individuals
• R.H.
(1) Group of 124. y = .082 + .3694 (x-1.177)
(2) R.H. y = .164 + .5719 (x-1.255)
PHOSPHORUS RETENTION IN GRAMS
PHOSPHORUS INTAKE IN GRAMS

Fig. 2 The regression of phosphorus retention on phosphorus intake.

intake, and indicated that, for this group, an intake of about 1 gm. was necessary for the women to maintain themselves in equilibrium while they were eating their customary diets.

For A.H. (Part II) phosphorus retentions were significantly dependent upon intake, as shown by a coefficient of correlation of 0.59. As with the large group, losses were more frequent at intake levels of less than 1.1 gm., than at the higher levels, the percentages of retentions increasing fairly consistently as intakes increased.

The regression of balance on intake for A.H. (fig. 2) indicates that an intake of about 1 gm. was necessary for her to maintain herself in equilibrium.

Mean daily phosphorus retentions of the group of nine women on the basic diet supplemented by 1, 2, and 3 cups of milk, respectively, did not increase with regularity as intakes increased. However, the amount retained was greatest at the 3-cup level. At intakes of less than 1.1 gm., mean retentions were larger and the percentage of retentions greater than was the case for the 124 individuals and for A.H. on their usual diets. At intakes of 1.1 gm. and above, no evidence of better retention by the group on the controlled diet was shown by the figures.

Nitrogen intakes

Mean daily intakes of the group of 124 ranged from 5.55 gm. to 17.58 gm. with a mean of 10.10 gm. which varied only slightly from the daily allowance of 60 gm. of protein recommended by the Committee on Food and Nutrition of the National Research Council (table 1).

The coefficient of variation (20%) was less than that for calcium or phosphorus and indicated less variability in nitrogen intake than was the case for either calcium or phosphorus.

For A.H. (Part II) the mean daily intake of 10.13 gm. was almost identical with that of the large group. A coefficient of variation of 8% indicated less variability from the mean than was the case for either calcium or phosphorus.

Mean daily nitrogen intakes of the group of nine women eating the controlled diets increased consistently as the level of milk intake increased, and ranged from 8.85 gm. to 13.51 gm.

Nitrogen retentions

Retention of nitrogen for the group of 124 individuals was significantly related to intake as shown by a coefficient of

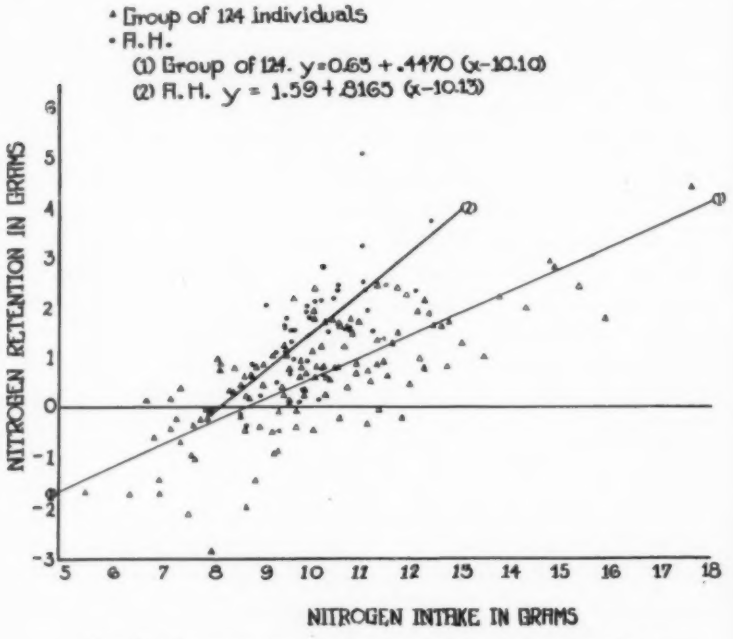

Fig. 3 The regression of nitrogen retention on nitrogen intake.

correlation of 0.73. At the lower levels of intake (less than 8 gm.) few retentions were observed. At intakes of 8 gm. and above, mean daily retentions as well as the percentage of the group storing nitrogen increased fairly consistently as intakes increased. Of the twenty individuals who had a mean intake of 12 gm. or more daily, all were storing nitrogen.

The regression of nitrogen balance on intake for the group of 124 indicated that at an intake of 8.69 gm., equilibrium might be expected for the group.

At mean daily intake levels of 9 gm. and above, A.H. stored nitrogen, the amounts stored increasing as intake increased. Regression of balance on intake showed that the intakes necessary for equilibrium for both the group of 124 and A.H. were similar.

Mean daily retentions for the nine women on the controlled diets increased slightly but consistently as milk intakes increased, but showed no evidence of better utilization of nitrogen than did the retentions for those women who were using their customary self-chosen diets.

Relative intakes and retentions of calcium, phosphorus and nitrogen

For the group of 124 women, coefficients of correlation of intake on retention were 0.50, 0.58 and 0.73 for calcium, phosphorus and nitrogen, respectively, and showed that phosphorus and nitrogen retentions were more closely dependent upon intake than was the case in regard to calcium. For A.H. (Part II) the coefficients of correlation for calcium and phosphorus intake on retention did not differ greatly, being 0.56 and 0.59, respectively, with a corresponding coefficient of correlation of 0.66 for nitrogen. A larger proportion of calcium than of either phosphorus or nitrogen is excreted in the feces. Because the marking of the feces is difficult, errors from this source tend to contribute to greater variation in calcium figures than in either phosphorus or nitrogen figures. This may have a bearing on the fact that retention of calcium, in contrast to the other two elements, appeared to be more dependent upon factors other than intake. This same source of error would operate similarly in all the studies.

At mean daily calcium intake levels ranging from 0.5 to 1.4 gm. there were (a) 106 studies of women on their self-chosen diets, (b) thirty-six studies for A.H., and (c) twenty-seven

studies for the nine women on the controlled diets. Of these, retentions were found for (a) 60% of the 106 studies of the women on their usual diets, (b) 61% of the A.H. studies, and (c) 78% of the studies of women on the controlled diets. These differences seem large enough to warrant the conclusion that the nature of the diet was a factor in calcium retentions and that the controlled diets had a stabilizing effect upon such retentions.

Milk, the food most logically used to increase the calcium intake, improves the diet also in factors other than calcium and tends, therefore, to improve calcium retention. Many young college women have diets which are far from adequate and undoubtedly this is also the case in regard to other young women. The desirability of a generous allowance of calcium for young women in general seems indicated by the findings of this study.

Phosphorus retention as related to calcium and nitrogen retentions.

Because the metabolism of phosphorus is related to that of nitrogen as well as of calcium, interrelationships of the three elements are of interest. It is usually assumed that in the adult body, 1 part of phosphorus combines with 17 parts of nitrogen and 2.18 parts of calcium, giving rise to the retention formula $P = \frac{Ca}{2.18} + \frac{N}{17}$ (Stearns, '33). By means of this formula, the phosphorus retentions were computed for (a) the group of 124 women, (b) A.H., and (c) the group of nine women. Computed retentions as compared to actual retentions were, respectively, as follows: (a) 0.051 and 0.062, (b) 0.127 and 0.164, and (c) 0.079 and 0.101.

These results of comparing actual and computed phosphorus retentions may indicate that a study of intakes and retentions of a large group of individuals, even though the periods of study are short, may show a better picture of the needs of individuals of corresponding ages than a more intensive study of a smaller group of individuals or of one individual.

Individual differences are minimized in the former case, whereas in the latter cases they tend to influence the results.

SUMMARY AND CONCLUSIONS

Calcium, phosphorus and nitrogen intakes and retentions were studied for (a) 124 college women from four states, on their customary self-chosen diets; (b) an individual for forty-four 5-day periods at intervals during her nineteenth through her twenty-second year; and (c) a group of nine young women eating basal diets supplemented with 1, 2, and 3 cups of milk, respectively. Results showed that place, age and weight differences were not significant for the 124 subjects, and that intake was significantly related to retention. Figures of regression of balance upon intake showed that mean daily intakes of 0.8 gm. calcium, 1 gm. phosphorus and 9 gm. nitrogen, respectively, were required for equilibrium. Corresponding figures for the one subject were 1.1, 1, and 8 gm.

That the type of diet was one of the factors influencing calcium retention was shown by the larger percentage of calcium retentions of subjects on controlled diets as compared with those on their customary self-chosen diets, when the calcium intakes were similar.

In view of the fact that diets probably vary among the general population to a greater extent than did those used by the group of college women studied, the desirability of a generous calcium allowance for young women of college age (17 to 24) seems indicated, and the calcium allowance of 1 gm. recommended by the Committee on Food and Nutrition of the National Research Council for girls of 16 to 20 might well be recommended for the group 17 to 24 years old.

For the large group of 124, calcium, phosphorus and nitrogen retention relationship was similar to the relationship commonly accepted. This was not the case for the individual whose balances were studied intensively. For the purposes of estimating population needs, it would seem that a short-time study of a large group is more desirable than the intensive study of one individual.

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SOYBEAN PROTEIN AS A SOURCE OF AMINO ACIDS FOR THE CHICK

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One of the most interesting characteristics of the soybean protein is the great increase in its nutritional value after sufficient heat treatment. The nature of the difference between raw and heated soybean protein has been shown to involve the availability of the sulphur-bearing amino acids. The literature on this topic has been thoroughly reviewed by Hayward and Hafner ('41). These authors concluded, on the basis of experiments with both chicks and rats, that their diets containing raw soybean protein were deficient in available cystine and supplied a positive but sub-optimal level of available methionine.

In all of the studies reported so far, the chick diets have contained appreciable amounts of protein other than that of the soybean. It is, therefore, not possible to attribute the results with these diets entirely to the soybean protein. An effort to avoid this difficulty was made by the use of a purified diet which contained no form of protein other than that furnished by the soybean or soybean meal. At the same time the now well-proven multiple function of methionine was given consideration. Methionine in the diet is the sole source of methionine in the animal tissues, but it may also furnish the sulphur for the synthesis of cystine and the methyl groups for the synthesis of choline and creatine. The methionine requirement for growth may, therefore, be affected by the presence or absence of dietary choline and cystine in so far

as these compounds in the diet may influence their rate of formation from methionine in vivo.

Studies with a few other amino acids were conducted to obtain further information on the completeness of heated soybean protein for the chick.

METHODS AND RESULTS

The basal diet employed in this work contained the following per 100 gm.: calcium gluconate 5.0, ox-bile salts 0.25, fish oil (400D–5000A) 0.25, tricalcium phosphate 2.0, dipotassium phosphate 0.5, potassium chloride 0.5, potassium iodide 0.001, sodium chloride 0.5, sodium silicate 0.2, manganese sulphate monohydrate 0.1, and magnesium oxide 0.2 gm. Water-soluble vitamins were added in the form of thiamine 1, riboflavin 1, pyridoxine 1, nicotinic acid 0.5 and calcium pantothenate 3 mg. An extract of brewer's yeast, prepared by leaching with 50% methanol and concentrating under reduced pressure, was added at a level equivalent to 5 gm. of yeast.

The variables in the diets were ground raw soybeans, commercial solvent-process heated soybean meal, pure amino acids, gelatin, soybean oil and glucose. The different forms of soybean protein were added to the diets in amounts to provide 20 gm. of crude protein per 100 gm. of diet as determined by analysis. Gelatin and amino acids, where added, were in addition to the soybean protein. Crude soybean oil in the amount of 5 gm. was added with the heated soybean meal. Finally, glucose was added to complete the 100 gm.

The chicks were Single Comb White Leghorn stock that had been reared for 1 week on a practical diet, then carefully selected for uniform weight and vigor and placed in experimental groups of eight chicks each. The chicks were housed in all-metal, electrically-heated battery brooders and given the diets and water ad libitum. Weighings were made at intervals of 3 to 4 days. The experimental period lasted from 10 to 14 days. The course of an experiment was almost invariably clearly indicated in the first few days. This has been generally true of chick amino acid studies.

The growth results are expressed in the form of average percentage gain per day. This is the average gain divided by the average weight of the chicks during the experiment and the time in days, and multiplied by 100. For the strain of chicks and the age period used, this percentage gain is close to 7.0 when the chicks are fed a practical rearing diet with approximately 20% crude protein.

The methionine content of the raw soybean protein was 1.9% and that of the soybean meal protein 2.1%. The gelatin used contained less than 0.1% methionine. The determinations of methionine were made by the method of McCarthy and Sullivan ('41). The diets used and the results are listed in tables 1 and 2.

DISCUSSION

A. Raw soybean protein

From the results of experiments with raw soybeans as given in table 1 the conclusion seems inescapable that lack of available methionine is the chief growth-limiting deficiency in unheated soybean protein.

The rates of gain observed in the case of raw soybean diets unsupplemented with methionine were from 2 to 3% per day.

TABLE 1

Supplements to the raw soybean diet.[1] *Relation of the supplements to the chick percentage gain.*

DIET NO.	CHOLINE CHLORIDE	l-CYSTINE	dl-METHIONINE	GELATIN	GLYCINE	PERCENTAGE GAIN PER DAY
1	0.10	...	...	6	...	2.8
2	0.40	...	...	6	...	2.1
3	0.10	0.20	...	6	...	2.8
4	0.10	...	0.25	6	...	5.9
5	0.10	...	0.50	6	...	6.9
6	...	...	...	..	0.50	3.1
7	0.10	...	...	..	0.50	3.2
8	0.10	0.16	...	..	0.50	3.4
9	0.10	...	0.25	5	...	6.6
10	0.10	0.20	...	5	...	2.0

[1] Expressed in per cent of diet.

The addition of choline (diets 6 and 7) or of l-cystine (diets 1 and 3, 7 and 8) produced little or no increase in this rate of gain. On the other hand, the addition of dl-methionine resulted in a striking increase in the rate of gain (diets 4, 5, and 9).

The rates of gain observed on the diets not supplemented with methionine, by comparison with similar rates obtained with purified diets containing arachin as the protein source (unpublished data), would indicate that methionine in the raw soybean protein is not entirely unavailable. The available quantity would seem roughly comparable to that in arachin; namely, approximately 0.6% of the protein.

The optimal level of supplementary dl-methionine for raw soybean protein appears to lie between 0.25 and 0.50% of the diet. This level permits a rate of gain which is comparable to that obtained on practical diets of the same total crude protein level; it is also equivalent or slightly superior to that observed in the case of heated soybean protein (table 2) without a methionine supplement.

B. Heated soybean protein

Because of the more practical aspects of the heated soybean protein, more experimentation was devoted to a study of the protein value of soybean meal made by a controlled heating process. The results given in table 2 show that this type of soybean protein, when unsupplemented, was capable of supporting rates of gain not far below the standard 7% (diets 11 and 23). The addition of gelatin did not appreciably affect this rate of gain. The addition of l-cystine (diets 17 and 18; 24 and 29) caused little or no increase in rate of gain.

The addition of choline (compare diets 12 and 13; 23, 24 and 25; and 26 and 27) resulted in small but definite increases in rate of gain. Choline chloride at a level of 0.4% of the diet appeared slightly detrimental to growth in each of three comparisons with a 0.1% level (diets 1 and 2; 13 and 14; 15 and 16). Choline was employed at this high level in order

to insure the presence of a sufficient amount of choline to methylate any homocysteine that might be present. A comparatively high level of choline is required for this process in the chick (Klose and Almquist, '41). The results obviously do not suggest the presence of any appreciable amounts of available homocysteine in either raw or heated soybean protein.

TABLE 2

Supplements to the heated soybean meal diet.[1] *Relation of the supplements to the chick percentage gain.*

DIET NO.	CHOLINE CHLORIDE	l-CYSTINE	dl-METHIONINE	l-LYSINE	l-TRYPTOPHANE	l-ARGININE	GLYCINE	GELATIN	PERCENTAGE GAIN PER DAY
11	...	...	...	...	...	...	...	..	6.5
12	...	...	...	...	...	...	...	6	6.6
13	0.10	...	...	...	...	...	...	6	7.2
14	0.40	...	...	...	...	...	...	6	6.7
15	0.10	...	0.13	...	...	...	...	6	7.8
16	0.40	...	0.13	...	...	...	...	6	7.4
17	0.10	...	0.13	...	0.10	...	...	5	7.7
18	0.10	0.28	0.13	...	0.10	...	...	5	7.6
19	0.10	0.28	...	...	0.10	...	...	5	7.1
20	0.10	0.28	0.13	...	...	...	...	5	7.9
21	0.10	0.28	0.13	...	0.10	0.40	1.00	..	7.4
22	0.10	0.28	0.13	0.30	0.10	0.40	1.00	..	8.1
23	...	...	...	...	...	...	0.50	..	6.5
24	0.10	...	...	...	...	...	0.50	..	7.0
25	0.20	...	...	...	...	...	0.50	..	7.2
26	...	...	0.20	...	...	...	0.50	..	7.6
27	0.10	...	0.20	...	...	...	0.50	..	7.9
28	...	0.16	...	...	...	...	0.50	..	7.3
29	0.10	0.16	...	...	...	...	0.50	..	7.3

[1] Expressed in per cent of diet.

The small but distinct growth-increasing effect of choline is probably explainable on the basis of a methionine-sparing action. It seems unlikely that the basal diets could have been deficient in choline, since it has been shown that soybean meal at levels appreciably lower than those employed in the present work is a fully effective source of choline for the chick (Jukes, '41).

That heated soybean protein is slightly sub-optimal in methionine content is shown by the fact (diets 13 and 15; 19 and 20; 24 and 27) that a distinct increase in rate of gain took place in every case when dl-methionine was added. If it is assumed that all of the methionine (2.1%) in the heated soybean protein is available and that the added dl-methionine is fully effective, the optimal dietary methionine level, in the presence of added choline and cystine, would be approximately 0.55%.

The results with diets 17, 18, 19 and 20 indicate that there is no appreciable deficiency of tryptophane in heated soybean protein as far as the requirements of the chick are concerned. Diet 21 as compared to diet 22 furnishes an indication of a slight lysine deficiency for the highest rate of gain; this indication, however, is not supported by the results with diets 26 and 27 which yielded very satisfactory rates of gain in the absence of a lysine supplement.

It is evident that all other amino acids required in the diet of the chick are well provided by heated soybean protein at a level of 20%. It is further evident that the non-protein portion of the meal must be well utilized by the chick, since the diets used in this work contained as much as 30% of the fat, carbohydrate, and other non-protein components of the soybean.

The high rates of gain observed in the case of many of the diets suggest that 20% of heated soybean protein plus a small supplement of methionine constitutes at least as good an amino acid source for the chick as is found in either a typical practical diet of the same total crude protein level, or in several combinations of other proteins in a similar purified diet (Almquist and Mecchi, '42).

The rates of gain obtained with the raw soybean protein supplemented with gelatin, choline and methionine fell slightly but distinctly short of the values obtained with correspondingly supplemented heated soybean protein. This would indicate an improved general plane of nutrition with the latter product, as suggested by Hayward and Hafner ('41).

While our results seem in some ways to differ from those of Hayward and Hafner, it should be recalled that their diets for chicks contained approximately 9% of soybean protein, either raw or heated, out of a total protein level of 20% or more. It is entirely possible that the non-soybean proteins in the raw soybean diet may have so affected the composition of the total dietary protein as to present the condition of a positive but sub-optimal level of methionine in the diet. Our results with raw soybean protein alone in the diet indicate that an acute methionine deficiency exists, while there is little or no evidence for an uncomplicated cystine deficiency.

SUMMARY

1. Raw and heated soybean protein were studied as the sole source of protein in a chick diet.

2. The principal growth-limiting deficiency in raw soybean protein is that of methionine.

3. Heated soybean protein is slightly deficient in methionine for the chick at the 20% protein level, but is complete in respect to all other amino acids required by the chick.

ACKNOWLEDGMENT

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BODY FATS IN RAT ACRODYNIA [1, 2]

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An accompanying report of this series (Quackenbush et al., '42) dealt with the multiple nature of the deficiency which causes rat acrodynia. The acute symptoms which developed in animals fed a diet free from unsaturated fat were cured either by linoleic acid or by the combined effects of pyridoxine, pantothenic acid and an unidentified water-soluble factor present in a rice bran concentrate.[3] While linoleic acid was unique in curing the acrodynia when fed alone, the water-soluble factors as a group cured the acrodynia and restored growth. However, for the complete cure of the chronic dermatitis, manifested only by a scaliness of the feet and tail, both the water-soluble factors and linoleic acid were required.

In view of these results the question arose whether the water-soluble factors cured acrodynia by altering some phase of fat metabolism; for example, by (a) promoting more efficient utilization of the unsaturated acids; (b) producing desaturation; or (c) effecting the synthesis of small amounts of linoleic acid or its physiological equivalent from carbohydrate or protein (Sinclair, '40). It seemed possible that changes in the course of fat metabolism would be revealed by a study of the amount and character of the fat present in the

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[2] The authors are indebted to Lever Brothers Company for a grant in support of this work.

[3] Vitab.

body. The view that water-soluble vitamins play a role in fat metabolism has been expressed by several workers. Thiamine and pyridoxine have been reported to affect the deposition of fat in the liver (McHenry, '37; Halliday, '38; McHenry and Gavin, '41). Pyridoxine has been suspected of functioning in the utilization of essential fatty acids (Birch, '38).

EXPERIMENTAL

Procedure

Animals were analyzed at different ages, viz., at weaning, at the time of development of severe acrodynia, and after the supplements had been fed for 3 weeks. The first two groups were normal weanlings produced on our stock diet, Diet S (Steenbock, '23), and a potato meal diet, Diet P (Quackenbush et al., '39), respectively. The third group consisted of acrodynic animals of uniform weight with a dermal index of 5 to 6 which had received the acrodynia-producing diet [4], Diet 5 (Quackenbush et al., '39), after having been raised to weaning age on diet P. Groups 4 to 8, inclusive, had the same history as the third group except that they had received test supplements as described in table 1 for 3 weeks.

TABLE 1

Status of rats when taken for analysis.

GROUP	BASAL DIET	SUPPLEMENT (DAILY FOR 3-WEEK PERIOD)	NO. OF RATS	AGE (WEEKS)	MEAN BODY WEIGHT	MEAN DERMAL INDEX
1	S		8	3	44	0
2	P		6	4	40	0
3	5	None	23	9–10	58	5
4	5	Pantothenic acid 50 μg.	6	12	50	13
5	5	Pyridoxine 20 μg.	10	12	59	6
6	5	Pantothenic acid 50 μg. Pyridoxine 20 μg.	5	12	82	3
7	5	Rice Bran Concentrate [1] 200 mg.	17	13	101	2
8	5	Ethyl linolate 10 mg.	4	13	56	1

[1] Vitab.

[4] Labor for the purification of the casein used in diet 5 was furnished by the Works Progress Administration.

The analyses consisted of determinations of amount and iodine values of body fat and liver fat. They were made by methods similar to those used by other workers. The rats were killed with ether, and the tissues then cut into small pieces and digested on a steam bath with 100 cc. of 30% alkali per 100 gm. of tissue. After 3 hours, one-fifth volume of alcohol was added and the digestion continued for 1 hour. The hydrolysate, freed from bones, was poured into a separatory funnel, cooled under the tap and acidified with dilute hydrochloric acid (1:1). The fatty acids were extracted with 100 cc. of freshly distilled petroleum ether per 200 gm. of tissue. The solution was washed with water, centrifuged, and the solvent removed under reduced pressure. Control tests with added oleic acid gave quantitative recoveries. Iodine values were determined by the method of Yasuda ('31).

Results of analyses

The data show that animals from diet S contained more than twice as much fat as those from diet P and, furthermore, that this fat was more highly unsaturated. These differences are of considerable significance in view of our earlier observations (Quackenbush et al., '39), that weanling rats when taken from diet P developed acrodynia in 4 to 5 weeks, while rats of approximately the same weight from diet S developed acrodynia in 8 to 10 weeks. In the former case the symptoms were acute; in the latter, the symptoms tended to assume the chronic form.

With the development of acute acrodynia the animals were found to have lost a large part of their body fat; however, the residual fat was more unsaturated than the original stores. This situation was not changed in 3 weeks by the feeding of either pantothenic acid [5] or pyridoxine.[5] However, when the two supplements were fed together, marked increases in weight and partial alleviation of the dermal symptoms were accompanied by a threefold increase in body fat, and a slight

[5] The pyridoxine, pantothenic acid and riboflavin were generously furnished by Merck and Company.

lowering of its iodine value. With a supplement of rice bran concentrate, which produced a greater growth response and cured the dermal lesions completely, the body fat was increased fivefold and the iodine value diminished to that of weanling rats on the potato diet. On the other hand, linoleic acid which produced no increase in body weight cured the acrodynia, increased the body fat and, curiously enough, decreased the iodine value slightly.

TABLE 2

Changes in tissue fats of rats with acrodynia.

GROUP	DESCRIPTION OF GROUP	CARCASS FAT ACIDS			LIVER FAT ACIDS			TOTAL UNSAT'D ACIDS (CALC'D AS OLEIC GM.)	UNSAT'D ACIDS % OF TOTAL
		Wt.	%	I.V.[1]	Wt.	%	I.V.		
1	Weanlings S	4.8	11.1	72	.13	5.2	134	4.03	82
2	Weanlings P	2.1	5.6	66	.16	6.6	94	1.65	72
3	Acute acrodynic	1.2	2.2	78	.08	2.3	98	1.14	86
4	Pantothenic acid [2]	1.2	2.3	76	.07	2.6	118	1.12	87
5	Pyridoxine	1.5	2.6	77	.07	2.0	107	1.34	85
6	Pantothenic acid and pyridoxine	3.9	5.0	74	.16	3.9	120	3.42	85
7	Rice bran concentrate [3]	5.9	6.2	67	.14	2.8	99	4.52	75
8	Ethyl linolate	2.0	3.5	73	.07	2.5	107	1.68	82

[1] Iodine value.

[2] Groups 4 to 8, inclusive, received the substance named in this column as a daily supplement for a 3-week period. See column three of table 1.

[3] Vitab.

In general, a high degree of unsaturation prevailed in the fats of acrodynic rats, and in all cases the lower values were those of normal groups, or groups which had been cured of acrodynia. This finding is open to a number of interpretations: (1) Since the acrodynic animal suffers from a deficiency of linoleic acid or the physiological equivalent commonly called "essential" fatty acid, it may tend to produce larger amounts of unsaturated fat in an attempt to supply its needs. (2) Pantothenic acid and pyridoxine may have effected a stimulation of the production of unsaturated acids; however, this would necessitate recognition of a counteracting effect of some other factor in rice bran concentrate. (3) As has been

shown by Sinclair ('35) certain tissues tend to hold tenaciously to the more highly unsaturated fatty acids during fasting or inanition. It is possible that the higher degree of unsaturation of the fat of pathological animals was a reflection of this tendency, and that the fat synthesis which followed the healing of the lesions and consequent improvement in general condition was of such a nature as to re-establish the normal degree of unsaturation of the body fat.

The trend of changes of the liver fats was similar to that of the body fats. Although some differences in percentages of fat were noted, none of the livers were fatty. Some increases in iodine values were apparent in animals which had received pantothenic acid, pyridoxine and ethyl linolate, but not in those which had received rice bran concentrate.

Non-curative effect of isolated fats on acrodynia

Since the available chemical methods seemed inadequate for the detection of small concentrations of essential fatty acids an attempt was made to determine the biological potency of the isolated fatty acids. Evans and Lepkovsky ('32) had shown that the methyl esters of fatty acids from normal rats were curative of fat-deficiency symptoms when fed in doses of 5 drops daily. Accordingly, the fatty acids from both the rice bran concentrate group (group 7) and from the acrodynic rats (group 3) were esterified. The esters from the former were fed to seven acrodynic rats, and those from the latter to three similar rats. No improvement resulted from the daily administration of 20 to 30 drops of either supplement; the severity of the symptoms increased and many of the animals died before the end of the 3-week assay period. It was apparent that little storage of essential acids had been effected by rats during the production of acrodynia, and that no marked synthesis had resulted from the feeding of rice bran concentrate.

SUMMARY

During the development of acrodynia in rats on a low-fat diet, the crude fatty acids decreased in amount but increased in iodine number.

The non-curative single supplements, pyridoxine and pantothenic acid, produced no significant alterations in the quality or quantity of fat.

Supplements which cured or alleviated the dermal symptoms, viz., linoleic ester, rice bran concentrate, or pyridoxine plus pantothenic acid, produced increases in total fat and decreases in iodine number.

Fatty acids from acrodynic rats or from rats which had been cured with rice bran concentrate did not cure acrodynia.

The studies did not reveal the mechanism through which pyridoxine, pantothenic acid and linoleic acid protect against acrodynia.

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NUTRITIONAL STUDIES ON POWDERED CHICKEN FEATHERS

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THREE FIGURES

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Recent studies from this laboratory (Routh, '42) have indicated that, when properly supplemented, powdered wool can be utilized by the rat for growth. A similar study of the nutritive properties of powdered hen's feathers has since been completed. This keratin is essentially a waste product in the poultry industry and interesting commercial applications might therefore emerge from the demonstration of its utilization by the animal organism.

EXPERIMENTAL

To insure uniformity, a large well-mixed batch of hen's feathers which had been carefully cleaned and defatted was used as the starting material. The feathers were reduced to a powder by the process previously described for powdered wool (Routh, '40). In vitro digestion with trypsin reached a maximum when the feathers had been ground by 400,000 revolutions of the ball mill. Several batches (300–400 gm. each) were therefore ground for this period and combined for use in the feeding tests. The powdered feathers thus prepared had an average percentage composition of nitrogen 15.52, sulfur 2.86, cystine 4.75 and ash 1.5.

The diets in which the feathers were incorporated had the following percentage composition: powdered feathers 15, starch 41.4, sucrose 15, agar 2, salt mixture (Hubbell, Mendel

and Wakeman, '37) 2.5, hydrogenated cottonseed oil [1] 19, cod liver oil 5, and choline hydrochloride 0.1. As a source of the vitamin B complex each animal received daily 40 mg. riboflavin, 40 mg. thiamine hydrochloride, 200 mg. nicotinic acid and 50 mg. of rice polish concentrate [2] incorporated into two pills fed approximately 12 hours apart.

Inasmuch as data in the literature seemed to indicate that the four amino acids found in suboptimal concentrations in wool were also low in feathers the need for these amino acids was tested by incorporating them in different combinations in the diets at the following levels: tryptophane 0.1%, methionine 0.6%, histidine (as the monohydrochloride) 0.4%, and lysine (as the dihydrochloride) 0.7%. These and other supplements replaced an equivalent amount of starch in the diet.

The powdered wool used for comparison was similar to that described earlier (Routh, '42) and the casein used as a supplement in some of the tests was a commercial product employed in other dietary studies in the laboratory.

The diets were fed ad libitum to young rats weighing between 44 and 71 gm. The rats were housed in individual false-bottomed cages. Food consumption and body weight were recorded every 4 days. Individual growth records are presented in figures 1–3.

RESULTS

Moderate growth was produced when tryptophane, methionine, histidine and lysine (diet O, fig. 1) were added to the basal diet containing the powdered feathers (15F). When any one of these four amino acids was omitted (as indicated by its initial letter T, M, H, or L, in fig. 1) growth failed to occur. A definite decrease in weight was observed on diet H from which the histidine was omitted. The lysine-deficient diet (L) failed to support growth in contrast to previous observations of fair growth on a similar diet containing

[1] Crisco.

[2] Ryzamin B from Burroughs-Wellcome Co., Tuckahoe, New York; riboflavin and thiamine through the courtesy of Merck and Co.

powdered wool instead of the powdered feathers (Routh, '42). This suggests that hen's feathers probably contain less lysine. The average gain on diet O was 1.4 and 1.5 gm. per day in the two series of animals.

Since casein is cheaper and is relatively rich in the four amino acids used as supplements, its supplementing capacity was tested by adding it to the diet containing powdered feathers at levels of 3, 5, and 8%. Its supplementing ability when fed at an 8% level (FC8, fig. 2) was little greater than

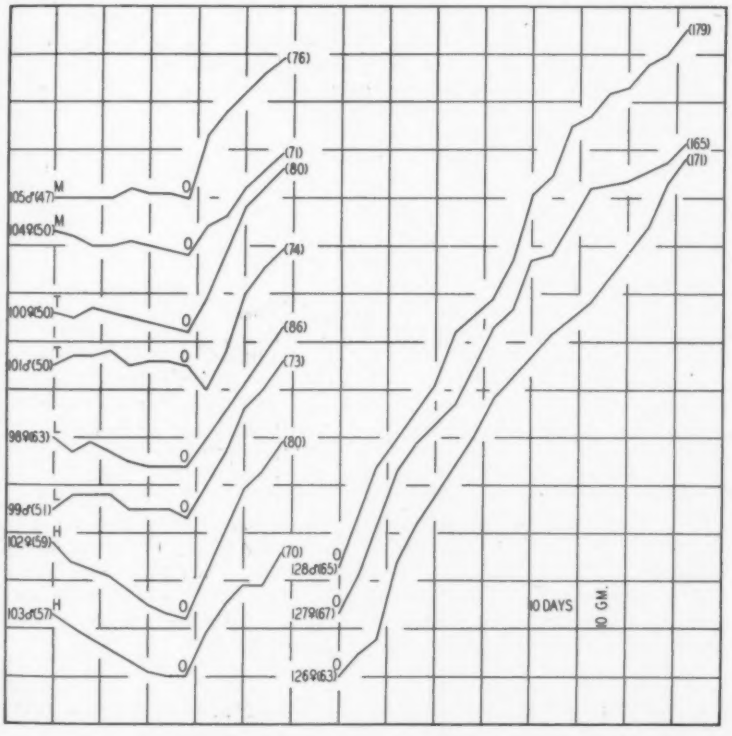

Fig. 1 Initial and final weights are given in parentheses. The various regimes and the food consumption per rat per day were as follows: O — diet 15F + 0.1% tryptophane, 0.6% methionine, 0.4% histidine, 0.7% lysine, 4.6 gm. (rats 98–105); 8.1 gm. (rats 126–128). M — diet O without the methionine, 3.0 gm. T — diet O without the tryptophane, 3.2 gm. L — diet O without the lysine, 4.1 gm. H — diet O without the histidine, 2.7 gm.

when fed at a 5% level (FC5, fig. 2). Average gains in weight were 1.5 gm. and 1.3 gm. per day, respectively. When it was fed at a 3% level (FC3, fig. 2) growth was decidedly inferior (only 0.4 gm. per day).

From a careful estimate of the amounts of each of the four amino acids present in the several diets it was concluded that diets FC5 and FC3 might still be deficient in histidine

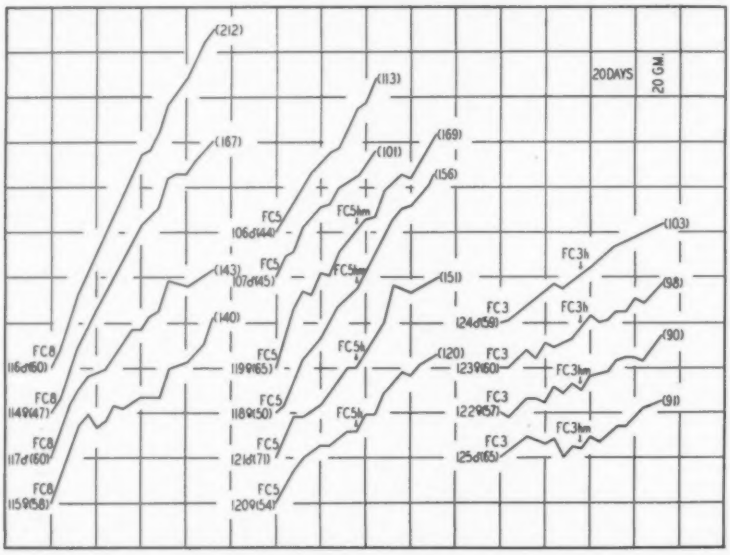

Fig. 2 Initial and final weights are given in parentheses. The various regimes and the food consumption per rat per day are outlined below. F is the basal diet containing 15% powdered feathers; C is casein and 3, 5 and 8 represent the per cent of casein; h is 0.15% histidine and m is 0.15% methionine. FC8 — 6.9 gm. (days 1–36), 7.8 gm. (days 37–72). FC5 — 6.3 gm. FC5hm — 8.0 gm. FC5h — 9.0 gm. FC3 — 4.8 gm. FC3h — 5.9 gm. FC3hm — 5.8 gm.

and possibly also in methionine. To determine the validity of this conclusion these diets were supplemented at the end of 36 days with either 0.15% histidine (FC 5h and FC3h) or 0.15% histidine plus 0.15% methionine (FC5hm and FC3hm) (fig. 2). These additions made little difference in the growth of rats on diet FC5 but increased appreciably the rate on diet

FC3. The average rate of growth on diet FC3 was 0.4 gm. per day and on FC3h and FC3hm 0.6 gm. per day. Since the growth rate did not approach that shown on the 5% level of casein it is obvious that there were also other limiting factors.

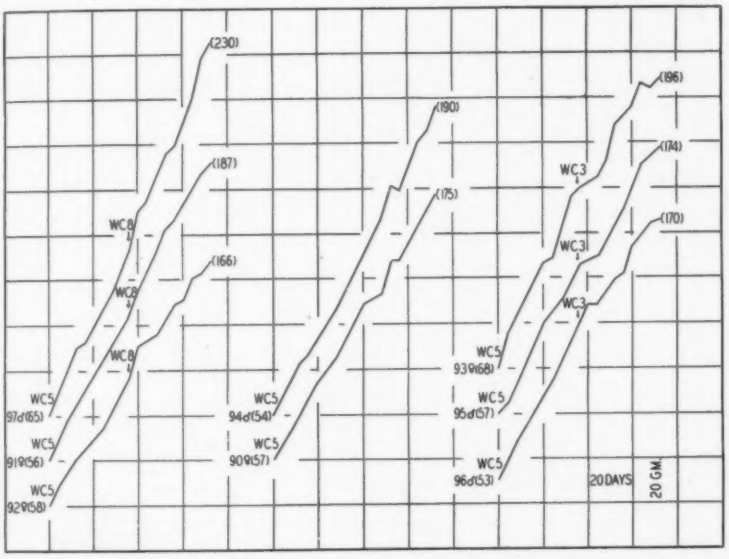

Fig. 3 Initial and final weights are given in parentheses. The various regimes and the food consumption per rat per day are outlined below. W is the basal diet containing 15% powdered wool; C is casein and 3, 5 and 8 represent the per cent of casein. WC5 — 7.1 gm. (days 1–36), 9.8 gm. (days 37–72). WC8 — 10.1 gm. WC3 — 9.0 gm.

The effect of supplementing diets containing powdered wool with casein is shown in figure 3. All of the animals in this group were placed on the basal diet containing 15% of powdered wool, modified by supplementation with 5% casein (diet WC5). After 36 days the supplement was changed to 3% casein or, in some instances, to 8%. The increase in weight of rats on these diets was more rapid than that observed in the rats on similar diets containing powdered feathers; the difference was especially striking on the diets containing 3%

of casein. The fact that wool is richer than feathers in histidine and lysine is probably responsible. Estimation of the amounts of these amino acids supplied by the diets suggests that histidine was the limiting factor. To judge from the similarity of growth rates, the utilization of the two powdered keratins was approximately the same in the diets containing 5 and 8% of casein.

The average food consumption on each diet is indicated in the explanation of the figures. In general it was roughly parallel to the growth rate.

Preliminary experiments with the hamster have given results very similar to those just described for the rat.

It appears that the protein requirement of domestic animals might be successfully met by feeding diets containing 15% of powdered feathers and 5% of casein; the commercial utilization of hen's feathers may thus be feasible.

CONCLUSIONS

Powdered hen's feathers are capable of supporting moderate growth in the young rat when they are supplemented with tryptophane, methionine, histidine and lysine and fed in a diet otherwise adequate.

When 5 or 8% of casein was used as a supplement instead of these amino acids the growth rate was essentially the same. When 3% of casein was used the rats grew only about one-fourth as rapidly.

Powdered wool supplemented with 5 or 8% of casein gave practically identical results; with 3% of casein the powdered wool diet produced a definitely higher growth rate than the diet containing the powdered feathers.

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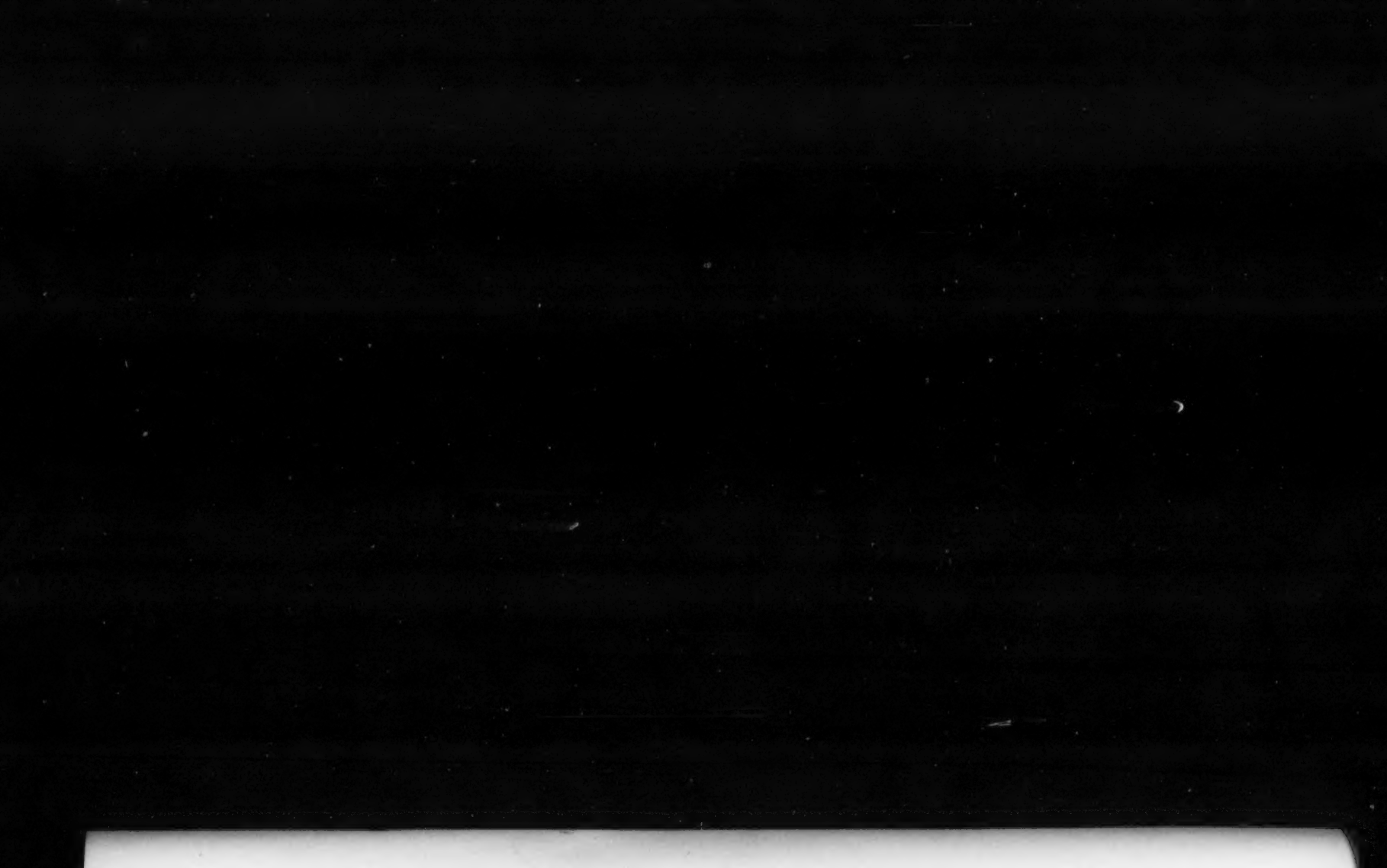
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